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7702 C*

Experimental Poliomyelitis in a Monkey without Demonstrable Lesions in the Central Nervous System.

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Experimental poliomyelitis is quite acute in the monkey, beginning as a rule with a high fever followed in 12 to 36 hours by cerebro-spinal fluid pleocytosis of approximately 25 to 100 cells, followed then by pre-paralytic symptoms such as irritability or apathy, excitement or inertia, ruffled hair, tremors and ataxia. This stage usually lasts for several to 24 hours, after which paralysis sets in, the cerebro-spinal fluid cell count increases up to 600 or 800 cells and then paralysis becomes complete. Histo-pathological examination of the central nervous system shows as a rule extensive nerve cell destruction and cellular infiltration.

In the course of our experiments on over 700 monkeys, one animal ran an unusually protracted clinical course, both in the preparalytic and paralytic stages. More unusual, however, was the absence of any histo-pathological lesions, diagnostic of poliomyelitis. However, an emulsion of this monkey's spinal cord produced the typical dis-

* P represents a preliminary, C a complete manuscript.

ease when injected into the monkeys, intracerebrally. These unusual findings seem to be of sufficient interest to report.

The monkey had the following clinical course:

May 11—Intracerebral inoculation of a partially neutralized virus-serum combination.

May 18—Temperature 103^{8}, slight head tremor, diarrhoea.

May 20— " 104^{3}, cerebro-spinal fluid—2 cells.

May 22— " 103^{5}, cerebro-spinal fluid—11 cells; globulin trace.

May 23— " 103^{7}, cerebro-spinal fluid—28 cells; differential count—polymorphonucler cells 10%, mononuclears 90%; globulin trace.

May 24—Temperature 102^{4}, cerebro-spinal fluid—116 cells, polymorphs 15%, monocytes 83%, bilobed cells 2%.

May 26—Temperature 103^{3}.

May 29— " 104^{8}—slight weakness of legs.

May 30— " 104^{5}—Same.

June 1— " 104^{7}—Increased weakness of legs.

June 2— " 103 —Legs still weaker; cerebro-spinal fluid—5 cells.

June 3— " 99—weakness arms; cerebro-spinal fluid—cells 5, globulin 0.

June 4—Greater weakness of the limbs, died during the afternoon.

The average normal temperature before the onset of poliomyelitis was 103^{4}F.

Histo-pathological study. Gross examination revealed a slight inflammation of the large intestine; the central nervous system showed nothing abnormal.

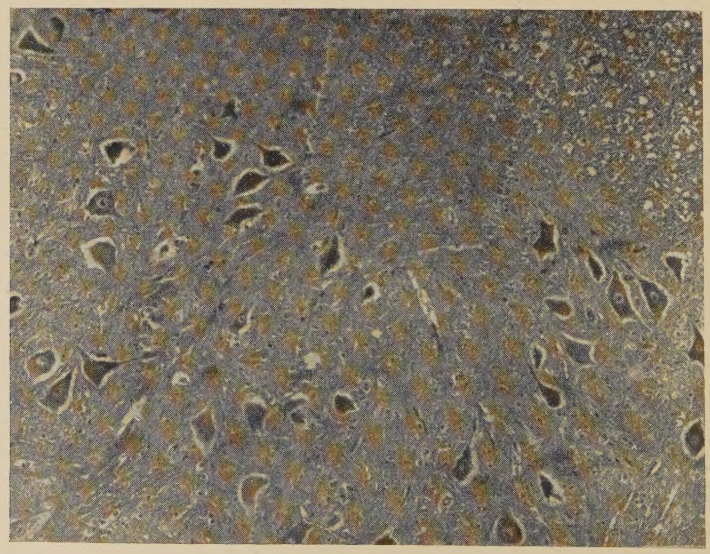

FIG. 1. Lumbar cord. Magnification 80—Phloxine—Methylene blue. No infiltration, oedema or nerve cell destruction.

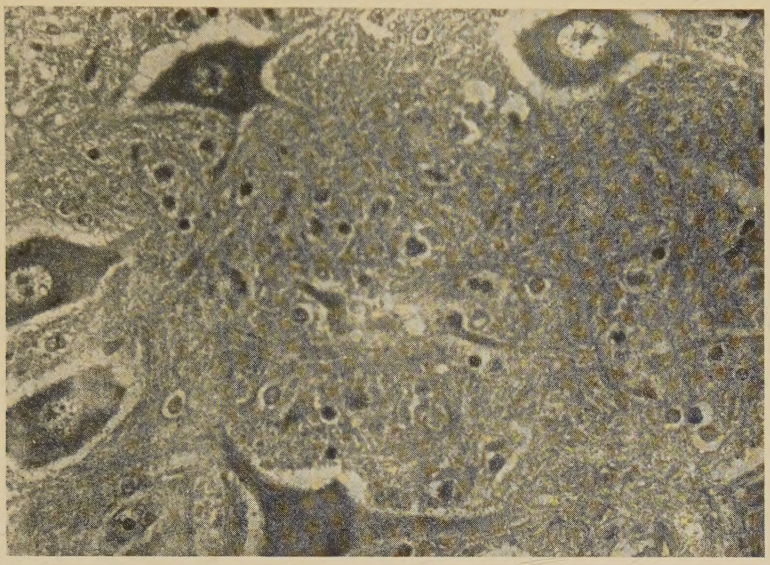

FIG. 2. Same. Magnification 360. Two small blood vessels without perivascular infiltration, no neuronophagia or satellitosis, no central chromatolysis, displacement of nuclei, edema or essential changes in any but one cell, which may be artifact.

Microscopic Examination. Examination of a number of selected blocks. The cord and brain stem were stained with hematoxylin-eosin and phloxine methylene blue. The meninges overlying the brain stem showed thickening with extravasation of red blood corpuscles, a few mononuclear cells, but elsewhere the subarachnoid spaces appeared to be entirely normal. The white matter showed no evidence of pathological changes.

The grey matter showed no hyperemia, perivascular or interstitial infiltration and no neuronophagocytosis or satellitosis. Except for the presence of a few rod cells and slight changes in some of the neurones such as central tigrolysis, shrivelling, pale or poor staining and occasional shift of the nucleus. These changes were in no way diagnostic of poliomyelitis and were found to a greater or lesser extent in monkeys that died of inter-current infection.

Although the clinical course progressed more slowly than usual, the head tremor, cerebro-spinal fluid pleocytosis and progressive weakness of the limbs were very suggestive of poliomyelitis. Moreover, the pathology outside of the central nervous system did not seem sufficient to account for the death of the animal. However, the histological findings were in no way similar to those of this condition. Nevertheless the spinal cord, when inoculated into other

monkeys gave the usual acute form of the disease with widespread nerve cell destruction and infiltration of the grey matter. On further serial passage the typical acute form was produced and the spinal cord showed the usual high infectivity. The spinal cord of the animal under discussion was infective in no greater dilution than 1:20 whereas a 1:8000 dilution of a suspension of the cords of animals running the typical acute form, gave infection.

Inasmuch as weakness developed during the last 5 days of a 10-day illness and was progressive up to the time of death, it appears that the attack of poliomyelitis was largely responsible for the death of the animal.

Of interest in this animal was the diphasic character of the attack. The first stage consisted of an increased temperature and tremors lasting 2 days, followed by a quiescent period of a week during which the animal appeared normal and then a second rise of temperature and progressive weakness. This simulates the human diphasic form described first by Medin[1] and Wickman[2] and stressed by Draper[3] (dromedary form) and although not uncommon in the experimental disease, the period of halt between the 2 phases is usually much shorter.[4] Since experimental poliomyelitis seems a disease of the central nervous system[5] exclusively the manifestations of the first stage must be due to an involvement of the upper part of the cerebro-spinal axis, possibly the thalamus, hypothalamus and midbrain; and the quiescent period due to halt of the virus in that region or its slow progress down the nerve tract until it reaches the anterior horn cells, giving the second attack. Thus, if in the experimental disease, which is entirely neurotropic, diphasic attacks occur, there is no need in the human disease to explain the first phase as due to systemic invasion, but rather to involvement of the central nervous system as Faber[6] has suggested.

Although elsewhere and in this laboratory experimental, less severe cases, with only mild grade histo-pathological changes, have been reported, in only one other instance has the virus of poliomyelitis been isolated from the spinal cord of a monkey that showed a suggestive clinical course but the absence of specific changes in the

[1] Medin, O., *Uerber eine Epidemic von spinaler Kinderlähmung Verland. d. X, Internat. Med. Kongr.*, 1890, 2.

[2] Wickman, I., *Nerv. and Ment. Dis. Monograph.* Series No. 16, 1913.

[3] Draper, G., Acute Poliomyelitis, Blakeston Son and Co., Phila., 1917.

[4] Brodie, M., and Wortis, B., *Arch. Neur. and Psychiatry,* in press.

[5] Brodie, M., and Elvidge, A. R., *Science,* 1934, **79**, 235.

[6] Faber, H. K., *Medicine,* 1933, **12**, 83.

cord. In this instance[7] the only apparent changes were vacuolization and sclerosis of the neuromes and possibly satellitosis.

These 2 cases remind me very much of Landry's[8] ascending paralysis. Therefore, I would suggest that when a case of ascending paralysis of unknown etiology and without any histo-pathological changes, as originally described by Landry and confirmed by Ormerod and Prince, Seifert, Rapper and others, or with very slight changes as described by Buzzard,[9] Williamson,[10] Stafford[11] and others, is encountered, the possibility of an unusual reaction to the virus of poliomyelitis be kept in mind and a piece of cord be removed, aseptically, for animal passage.

Conclusions. 1. A *Macacus rhesus* monkey infected with the virus of poliomyelitis ran an unusually slow course, which simulated the diphasic type found in the human. 2. The histo-pathological findings of the cerebro-spinal axis were essentially normal, but the diagnosis of poliomyelitis was made by successful passage of cord emulsion into other monkeys.

7703 P

Extraction of an Emulsion-Stabilizing Substance from Nitella with Distilled Water.

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Nitella cells, kept in distilled water for 3 days,* lose their irritability and their characteristic behavior with potassium.[1] This is apparently a result of the extraction from the cell surface of some organic substance or substances, which we may designate as *R*. Presumably *R* is constantly produced by the normal metabolism of the cell and accumulates in the cell surface if the cell is bathed by a

[7] Kling, C., Pattersson, A., and Wernstedt, W., Epidemic Infantile Paralysis, Report from the State Medical Institute of Sweden to the XV International Congress of Hygiene and Demography, Washington, 1912.

[8] Landry, *Gazette Hebdomodaire de Médecine et de Chirurgie,* 1859.

[9] Buzzard, E. F., *Brain,* 1907, **80**, 77.

[10] Williamson, R. T., Diseases of the Spinal Cord.

[11] Stafford, J. S., *Lancet,* 1915, **1**, 1172.

* This exposure to distilled water produces no sign of injury and such cells live indefinitely when transferred to pond water.

[1] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.,* 1933, **17**, 87, 99, 105.

balanced solution. In distilled water, or dilute solutions of alkali chlorides, *R* is dissolved from the surface more rapidly than it enters it.

Can the giving off of substances be detected? Groups of 5 *Nitella* cells were stored in 10 cc. of the solutions indicated in Table I, in

TABLE I.

10 cc. of solution was placed in each 15 cc. glass stoppered tube. The solutions in Tubes, 3, 6, and 9 were saturated with $CHCl_3$ before adding the cells.

After 3 days the cells were removed, 3 cc. of $CHCl_3$ added and the tube well shaken. The sign + indicates the presence of a substance (or substances) which stabilizes the emulsion; the sign — indicates its absence.

Tube No. 1.	H_2O	—
2.	H_2O + 5 *Nitella* cells	+++
3.	H_2O + 5 *Nitella* cells + $CHCl_3$	+
4.	Solution A	—
5.	Solution A + 5 *Nitella* cells	—
6.	Solution A + 5 *Nitella* cells + $CHCl_3$	+
7.	0.001 M $CaCl_2$	—
8.	0.001 M $CaCl_2$ + 5 *Nitella* cells	—
9.	0.001 M $CaCl_2$ + 5 *Nitella* cells + $CHCl_3$	+

15 cc. stoppered test tubes. After 3 days, the cells were removed, 3 cc. of $CHCl_3$ added to each tube, and the tubes vigorously shaken. In Tubes 1, 4, 5, 7, and 8 the emulsion broke up in a few minutes, the water and $CHCl_3$ forming discrete layers. In Tubes 3, 6, and 9 the $CHCl_3$ settled in large drops, which coalesced in the course of about 1 hour. In Tube 2, which had contained distilled water and *Nitella* cells, the $CHCl_3$ layer was broken up into many very small drops, and the emulsion persisted for days.

It is evident that cells which show no signs of injury can give up to distilled water substances which stabilize an emulsion of $CHCl_3$ and water. This action is very much less when the cells are bathed by calcium solutions or a balanced solution such as Solution A.† More of *R* is given up to distilled water by intact *Nitella* cells in 3 days than can be extracted from the killed cells, thus supporting the idea that *R* is constantly produced and given off.

† For the composition of Solution A, a balanced solution, see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933, **17**, 87.

7704 P

Directed Roentgenography of the Thorax.

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By a directed chest exposure is meant a roentgen exposure of the chest at any phase of the cardiac cycle. This is accomplished by the synchronization of the exposure with a definite phase of the circulatory phenomena, heart sounds, pulse wave or electrocardiographic impulse.

McPhedran and Weyl[1] found the use of the heart sounds unsatisfactory for this purpose because of interference of the extraneous sounds, but utilized the pulse wave with success. A cannula is applied over the carotid artery and the pulse thrust is transmitted to a tambour. A light beam reflected from a mirror on the tambour enters a photoelectric cell at the appropriate point in the cardiac cycle. The photoelectric current is amplified and actuates the X-ray switch after an appropriate delay. The sudden outward thrust of the carotid pulse occurs simultaneously with systole (mechanically), and in order to obtain an exposure at the end of diastole, a delay of slightly shorter duration than the cardiac cycle is necessary. The method is, therefore, not feasible when there is an irregularity of rhythm.

Meek and Eyster[2] used the electrocardiograph as a control. They closed the X-ray switch manually by estimating the appropriate point from the pulse wave and checked the result by noting the disturbance produced by X-ray exposure in simultaneously recorded cardiogram. Thus it was possible to state at what point in the cycle any particular exposure had been taken, but this method of timing cannot be very accurate and there must of necessity be many exposures which are not properly timed.

Our method makes use of the cardiac action current. This method was considered by McPhedran and Weyl and discarded by them as being too cumbersome. As developed by us, we believe it to be simpler and more effective than any method based upon the pulse.

The practical details are essentially these. The patient's leads are connected to those points which by means of a previous electrocardiogram have been found to yield a high erect or inverted R wave

[1] McPhedran and Weyl, *Radiology*, 1928.

[2] Meek and Eyster, *Am. J. Roentgenology*, 1920, **7**, 471.

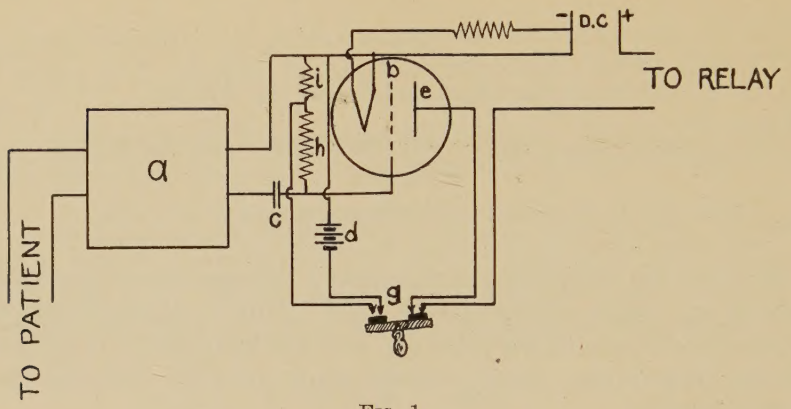

FIG. 1.

(over one millivolt and well above all other peaks of the electrocardiogram). The voltage thus led off is amplified two or three thousand times by means of a 2-stage amplifier (a in Fig. 1), the output of which is connected to the grid of a thyratron tube (b), through a condenser (c). As long as this condenser is connected to a charging battery (d), through the resistance (h), the grid is held so far negative that no discharge can occur. When all is ready for the exposure, the charging battery is disconnected and at the same time, the thyratron plate (e) is connected to a source of D.C. voltage through a relay. This double switching operation is accomplished by means of a switch as indicated diagrammatically at (g). The condenser now discharges through appropriate resistances (h and i), with a time constant of about 2 seconds, raising the grid potential until one of the high cardiac voltage pulses brings it over the critical voltage. The thyratron then becomes conducting and the X-ray contact is made by the relay, which by virtue of the properties of the thyratron now stays closed until the circuit to the plate is interrupted. Any suitable timer may be used, although we have found an arrangement based upon the charging of a condenser by the X-ray tube current to be most satisfactory.[3] Time delay devices may be inserted between the thyratron and the X-ray switch to make the exposure at any predetermined time after the R wave.

By means of an arrangement of relays, we have been successful in obtaining 2 pictures on the same film of the heart in 2 different selected phases of the same cardiac cycle. By this means we expect to investigate the relationship between the amplitude of heart motion, as shown by differences in the size of the heart shadow, and cardiac stroke output.

Further study will be necessary to determine the optimum point

[3] Schwarzchild, M. M., *Radiology*, 1930, **15**, 132.

of the cardiac cycle at which to expose roentgenograms in order that the unsharpness due to cardiac action may be minimized. Such study is in progress and involves the simultaneous recording of electrocardiogram and roentgenokymogram by a method similar to that used by us for the study of heart sounds.[4]

7705 P

Clinical Identification and Measurement of Urinary Sugars.

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In the routine urinalysis of the Prudential Laboratory all copper-reducing urines have for some years been measured by our modification[1] of Sumner's[2] di-nitro-salicylic acid method. Because of its speed, accuracy and independence of personal equations of technicians we use the Photo-Electric Scopometer,[3] but the method also works well with Junior Scopometer,[4] colorimeter or even permanent test tube standards.*

After Lasker and Enklewitz[5] drew attention to the fact that urines containing keto-pentose reduce Benedict's qualitative copper reagent at room temperature, we observed that ketose-containing urines also reduced our di-nitro-salicylic acid reagent at room temperature, perhaps more perceptibly than they do copper. When the reductions of di-nitro-salicylic acid by keto-pentose at room temperature were measured and the tests then boiled and the total reductions measured in our usual way, the results proved very consistent. We therefore began placing all di-nitro-salicylic acid tests in a water bath at 30° for 5 minutes and measuring whatever reduction might occur before going on in our usual way to boil the tests and measure the total reduction.

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[4] Hirsch and Schwarzchild, *Acta Radiologica*, 1934, **15**, 2, 84, 100.

[1] Rose, A. R., Schattner, F., and Exton, W. G., *Tr. Assn. Life Insurance Med. Directors of America*, 1929, **16**, 178.

[2] Sumner, J. B., *J. Biol. Chem.*, 1925, **63**, 393.

[3] Exton, Wm. G., *Am. J. Clin. Path.*, 1932, **2**, 411.

[4] Exton, Wm. G., *J. Am. Med. Assn.*, 1929, **92**, 708.

* Procurable from the Standard Reagents Co., 1709 Colonial St. (W. Oaklane), Philadelphia, Pa.

[5] Lasker, Margaret, and Enklewitz, Morris, *J. Biol. Chem.*, 1933, **101**, 289.

One result of this new step was the discovery of more pentose-containing urines than were hitherto seen. Another result was the finding of a constant proportionality between the ketose reduced at the lower temperature and the total ketose reduced by boiling: a relationship which seems to hold regardless of the concentration of ketose in the specimen.

This information, coupled with observations made in the course of previous experiments with other sugars and reagents, led us to inquire how urinary sugars other than ketose would behave under the same or similar conditions such as picric acid reductions, etc. The results of some of these studies are shown in Fig. 1, and similar results may, of course, be had with temperature constant and time variable.

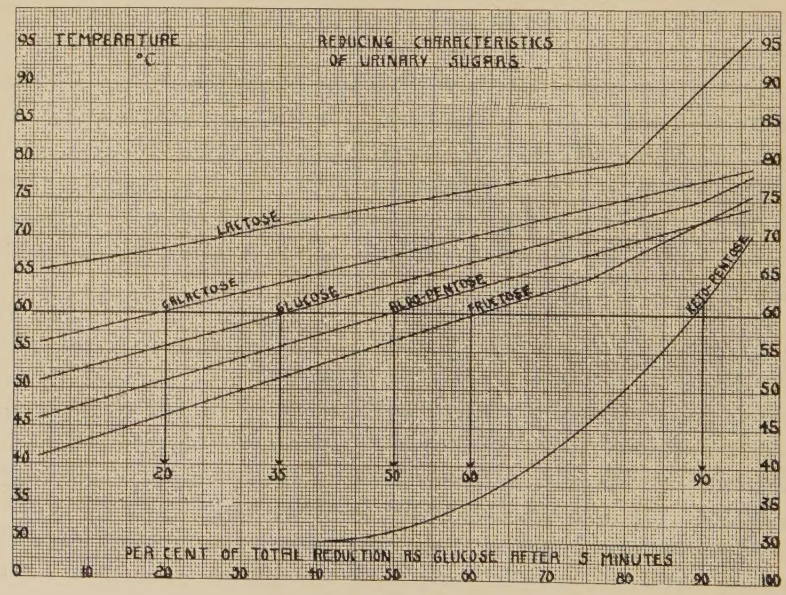

FIG. 1.

Working with a sensitive method that is accurate to 1%, and with temperatures thermostatically controlled within a degree, it is apparent from the data that the differences between the reductions of the various sugars at the higher and lower temperatures are great enough to enable the identification of any one of them. In fact, it is practicable to identify more than one sugar by additional observation and calculation.

In our routine urinalysis we therefore now measure the reductions

of all indicated specimens at a lower as well as at the higher temperature and carry out the procedure as follows:

We apply our di-nitro-salicylic acid method to all urines. This step eliminates the non-reducing urines which are reported sugar-negative and also provides the total reduction value of whatever reducing substances a specimen may happen to contain.

To all the reducing urines we then apply an improved (unpublished) phenyl-hydrazine test. This step eliminates all the non-sugar-reducing urines, which are also reported negative for sugar.

If phenyl-osazone crystals are present, another di-nitro-salicylic acid test is run at the lower temperature on a sample which has been adjusted so that concentrations of sugar are always 0.4%. This adjustment is readily accomplished by increasing the amount of or diluting the urine, as may be indicated by the known total reduction value of the sample. A simple calculation then gives the desired qualitative and quantitative information.

It will be observed that this simple addition to routine technics need be applied only to those specimens which are definitely known to contain some kind of sugar. It may also be noted that the actual manipulations of the new step take about 2 minutes, exclusive of time in the water bath, and that the complete procedure requires less than 2 cc. of urine.

7706

Absence of Dietary Anti-Anemia Substance in the Diet Causative of Canine Black Tongue.

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Goldberger[1] showed that autoclaved yeast possessed the power of preventing black tongue in dogs, and later that the liver fraction of Cohn and Minot,[2] which was therapeutically effective in pernicious anemia, was also prophylactic against black tongue. Autoclaved yeast was found by Strauss and Castle[3] to be capable of effecting remissions in pernicious anemia after it had been incubated with normal human gastric juice. The therapeutic similarity between

[1] Goldberger, J., and Sebrell, W. H., *Pub. Health Rep.*, 1930, **45**, 3064.

[2] Cohn, E. S., McMeekin, T. L., and Minot, G. R., *J. Biol. Chem.*, 1930, **87**, 49.

[3] Strauss, M. B., and Castle, W. B., *New England J. Med.*, 1932, **207**, 55.

canine black tongue and pernicious anemia suggested that the effect of the diet producing black tongue was due to a lack of that dietary constituent which is required for normal hematopoiesis in the human being. It has been found possible to test and substantiate this hypothesis by experiments on guinea pigs fed a diet which is causative of black tongue in the dog.

Adult guinea pigs weighing from 400 to 600 gm. were used. The animals were kept under uniform conditions with shavings as bedding. The Goldberger black tongue-producing diet was fed daily, and the animals were allowed to eat as much as they desired. The diet was composed of the following ingredients:

White corn meal,	6,000 gm.	Cotton seed oil	450 cc.
California black-eyed peas,	750 "	Calcium carbonate,	45 gm.
Casein (leached),	900 "	Sodium chloride	15 "
Cod liver oil,	225 cc.		

In addition to the special diet, each experimental animal was fed daily 2 cc. of fresh orange juice to ensure an adequate intake of vitamin C. The materials which were tested for their value in preventing the effect of the diet feeding were mixed with water and fed daily with a pipette.

TABLE 1.

Exp. No.	Therapy	No. animals	Aver. wt. beginning of exp. gm.	Aver. wt. end of exp. gm.	Aver. change per animal gm.	Result
1	Controls Diet alone	5	450	325	—125	Died during 2nd and 3rd weeks
2	Liver extract Lilly No. 343 0.6 gm. q. d.	5	415	455	+40	Survived
3	Vegex 1 gm. q. d.	4	457	366	—91	Died during 2nd and 3rd weeks
4	Vegex 2 gm. q. d.	3	393	426	+33	Survived
5	Ventriculin 1 gm. q. d.	4	408	310	—98	Died 2nd-5th week
6	Ventriculin 2 gm. q. d.	3	393	268	—125	Died during 3rd week
7	Vegex 1 gm. + Ventriculin 1 gm. q. d.	4	388	425	+37	Survived

The results are presented in Table I. All of the animals fed only the vitamin C supplemented diet producing black tongue lost weight rapidly and died before the end of the third week. When a daily supplement of 0.6 gm. of liver extract No. 343 was administered, all the animals survived and showed a gain in their average weight.

A commercial yeast extract sold under the name of Vegex was found to be ineffective when 1 gm. daily was fed, but was prophylactic when 2 gm. daily were used. This same extract was employed by Strauss and Castle[3] as a source of the dietary anti-anemia factor. Desiccated hog stomach given in 2 gm. amounts daily as Ventriculin was found to be ineffective, as would be expected if it contained only the gastric without the dietary anti-anemia factor. If 1 gm. of Ventriculin plus 1 gm. of Vegex were administered, however, complete prophylaxis occurred, although neither substance was effective alone in the dosage given. The results suggest that the deficiency which is causative of canine black tongue is closely allied to the deficiency which is etiologic in pernicious anemia. Moreover, the death or survival of the guinea pig fed the diet producing black tongue may serve as a useful test for evaluating the potency of various substances used in the treatment of pernicious anemia in the human being.

Conclusion. Guinea pigs fed a diet causative of canine black tongue lose weight rapidly and die within a short period. This effect may be prevented by substances which are capable of causing remissions in pernicious anemia.

7707 C

Multiplication of Equine Encephalomyelitis Virus in Mosquitoes.

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While Davis, Frobisher and Lloyd[1] failed to find evidence of multiplication of yellow fever virus in infected *Aëdes aegypti,* the experiments of St. John, Simmons and Reynolds[2] and of Holt and Kintner,[3] though limited in number, suggest that the dengue fever virus does multiply in its transmitting insect host. We have reported[4] that *Aëdes sollicitans* fed on animals infected with the

1 Davis, N. C., Frobisher, M., Jr., and Lloyd, W., *J. Exp. Med.*, 1933, **58**, 211.

2 St. John, J. H., Simmons, J. S., and Reynolds, F. H. K., *Am. J. Trop. Med.*, 1930, **10**, 23.

3 Holt, R. L., and Kintner, J. H., *Phil. J. Sci.*, 1931, **46**, 593.

4 Merrill, M. H., Lacaillade, C. W., Jr., and Ten Broeck, C., *Science*, 1934, **80**, 251.

eastern strain of equine encephalomyelitis virus show, by titration of the suspension of a given number of insects, a greater concentration of virus at 5 days than immediately after feeding. Titration experiments are not absolutely convincing and we therefore adopted a modification of the method used by St. John, Simmons and Reynolds in order to determine whether the equine encephalomyelitis virus could be carried by serial passage through mosquitoes. If it can be carried from mosquito to mosquito directly the proof of the multiplication of the virus is convincing.

Thirty female *Aëdes aegypti* infected 5 days previously by feeding on brain virus of the western strain of equine encephalomyelitis were suspended in 4 cc. salt solution plus 1 cc. normal horse serum. An equal amount of defibrinated horse blood was added and a pledget of cotton in a Petri dish was moistened with the mixture. A small amount of sugar was sprinkled over the surface of the cotton and the Petri dish was placed in a cage containing female *Aëdes aegypti* that had had no sugar solution for 4 days and no water for one day. Since the virus deteriorates rapidly when in contact with the air at room temperature, the Petri dish was replaced in an hour's time by one containing the mixture that had been kept in the refrigerator. After another hour this was removed, so that the mosquitoes that fed took up active virus. Those that did not feed were eliminated by withholding water for 24 hours and sugar solution for 48 hours from the entire lot. The infected mosquitoes were kept in cages at a room temperature of from 24-28°C.

At 6 to 7 day intervals from 25 to 30 mosquitoes from the last feeding have been suspended and fed to starved females as outlined above. At each transfer virus has been demonstrated in the suspension of crushed mosquitoes by guinea pig inoculation and in many instances dilutions as high as 10^{-5} have proven infectious. Control inoculations of 3 kinds into guinea pigs have all been negative: a suspension of mosquitoes from our healthy stock; the horse serum and saline used; and a boiled suspension of infected mosquitoes. Since the virus has now been passed in series through 10 lots of mosquitoes and since the dilution at each transfer is at least 1:100 we must conclude that multiplication has taken place.

No difference has been demonstrated between the mosquito passage virus and the original strain. Its serological characters are unchanged, the virulence has been modified little if at all, and it passes Berkefeld "N" filters readily. Mosquitoes infected with the passage strain readily infect guinea pigs by biting.

The virus seems to be generally distributed in the bodies of the

mosquitoes, for it has been demonstrated by guinea pig inoculation in suspensions of legs removed from uncrushed insects, as well as in suspensions of the body fluid, heads, thoraces, and abdomens. Since this virus kills horses and other mammals so readily, we might expect that a general invasion of the mosquito would likewise be fatal. This is, however, not the case, for the mortality in the cages containing infected mosquitoes is no higher than in those containing normal ones.

7708 C

Injections of Combined Paratyphoid Colon Bacillus Filtrate and Poliomyelitis Virus by Way of Gastrointestinal Tract.*

JOHN A. TOOMEY.

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Poliomyelitis was produced in monkeys by injecting saline suspensions of virus into the intestine between clamps or subserosally.[1] What effect would the addition of paratyphoid colon bacillus culture filtrate have on weak suspensions of virus if introduced in the same fashion?

Monkey I was injected directly into the intestines between intestinal clamps with 40 cc. of a 2% suspension of poliomyelitis virus and 40 cc. of P. C. B.† filtrate. The first day after injection, the animal had furring and weakness of the right foot; it was very sick and obviously paretic on the 3rd day. By the 5th day, both feet were weak, the right leg showed paresis, and the extensors of the right hand were weak. It recovered and on the 17th day was active again, although it still had some paresis with beginning atrophy of the muscle groups described.

Monkey II, control for monkey I, was injected directly into the intestines between intestinal clamps with 40 cc. of a 2% suspension

* Expenses defrayed in part by a grant from the Marion R. Spellman Fund, The Cleveland Foundation.

1 Toomey, John A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1015.

† A number of paratyphoid colon organisms listed in a previous communication[2] were planted in glucose broth, grown for 10 days and the material passed through an N and W filter. For convenience, I will term the paratyphoid colon bacillus culture filtrate, the P. C. B. filtrate.

2 Toomey, John A., *J. Infect. Dis.*, 1934, **54**, 74.

of virus and 40 cc. of normal physiological saline. Unlike monkey I, this animal always was active even though it had developed slight weakness of the left foot and leg on the 6th day after injection.

Monkey III was injected with 50 cc. of material. This dose was made up of 25 cc. of a 2% suspension of virus plus 25 cc. of P. C. B. filtrate injected subserosally at multiple points. Twenty-four hours after the injection the animal had some slight weakness of the muscles of both feet. In 2 days, both legs were definitely paretic and the hands were weak. In 5 days quadriplegia developed and in 8 days death occurred.

Monkey IV, control for monkey III, was injected with 25 cc. of a 2% suspension of virus plus 25 cc. of saline subserosally at multiple points. The animal remained active until the 15th day, although on the 7th day it had some paresis of the right foot. On the 15th day, it developed paresis of the left foot, leg and thigh, right foot and both hands. Although it recovered, some weakness and atrophy of the feet remained.

Monkey V was given 50 cc. of material. This dose was made up of 25 cc. of a 1% suspension of virus plus 25 cc. of P. C. B. filtrate injected subserosally at multiple points. In 24 hours, there was furring, the animal was sick and had paresis of the right foot. In 2 days, it had more obvious paresis of the right foot and thigh and left leg. In 5 days, it had paralysis of both feet and the right hand, and paresis of both legs and right thigh.

Monkey VI, control for monkey V, was given 50 cc. of material. This dose was made up of 25 cc. of a 1% suspension of virus plus 25 cc. of normal saline injected subserosally at multiple points. The animal was active at all times and never became sick or had furring, although there was some paresis of the left leg on the 7th day and weakness of the left thigh and right foot on the 15th day.

The P. C. B. filtrate, or enteric toxin, as it was previously called, was not toxic for monkeys. Three or more ounces of the filtrate was passed by stomach tube in 2 monkeys. There was no effect other than diarrhea and anorexia which lasted for 2 days. Two other animals were each injected with 15 cc. of filtrate directly into the femoral vein with no effect. An abdominal section was performed on 1 animal and 100 cc. of undiluted enteric toxin was injected through a needle directly into the small intestine. No paralysis developed. Two other animals were injected subserosally with 50 cc. of material consisting of 25 cc. of P. C. B. filtrate and 25 cc. of saline. No paresis or paralysis developed. Even the injection of

this P. C. B. filtrate directly into the nerve in the amounts mentioned caused no paralysis.[1]

Conclusion. These experiments indicate that the production of poliomyelitis is accelerated or intensified when poliomyelitis virus is combined with P. C. B. filtrate and injected subserosally or directly into the gastrointestinal tract. Two other sets of 2 animals each subsequently experimented upon in the same manner gave the same results.

7709 C

Comparative Study of Effects of Preparations of Posterior Lobe of Pituitary Gland on Water Interchange in Frogs.

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One of us (Steggerda[1]) demonstrated that frogs given injections of pitressin absorbed considerable amounts of water in which they were kept, an increase of nearly 15% of body weight being reached in from 4 to 5 hours. Experiments in which the cloaca was tied off to prevent elimination of water and experiments similar to those described by Adolph[2] in which a comparison was made between frogs used as controls and frogs without skins led to the conclusion that pitressin has a specific effect on the permeability of the frog's skin.

With these results in mind we carried out experiments comparing the effects of solution of pituitary U.S.P. (we used pituitrin, Parke-Davis), and its separate components, pitressin, and pitocin, respectively, on the absorption of water by the frog, *Rana pipiens.* The results are here presented. We shall also report our observations on the localization of the absorbed water, and present data on the influence of pitocin on the rate of loss of water by the frog.

At the beginning of each experiment frogs weighing 40 to 50 gm. were placed in a container and nearly submerged in water at room temperature. After 30 minutes the frogs were removed, dried with gauze as uniformly as possible, and weighed on a beam balance accurate to 0.1 gm. An amount of pituitrin, pitressin, or of pitocin

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[1] Steggerda, F. R., *Am. J. Physiol.*, 1931, **98**, 255.

[2] Adolph, E. F., *Am. J. Physiol.*, 1931, **96**, 569.

equivalent to 0.1 cc. for each 10 gm. of body weight was then injected into the dorsal lymph sac of each frog and the frogs were again placed in the water. This same general procedure was followed for the frogs used as controls except that they did not receive the injections of the preparations of posterior pituitary. Weighings were made at intervals of approximately 30 or 60 minutes. Four sets of observations were made with each preparation; in each case 3 frogs received the injections and one was kept as a control. A total of 12 frogs therefore received each pituitary product. The values referred to in the following statements represent averages of all the observations with each preparation. The experiments were carried out during July and August.

Each of these posterior pituitary products caused a very definite increase in weight. The maximal increase following injections of pituitrin or pitocin was reached in from 3 to 4 hours, whereas pitressin produced its maximal effect in about 2 hours. Pitocin produced the most striking effect, since injections of this preparation caused a maximal increase in weight of about 19% in contrast to a maximal increase of about 16% for injections of pituitrin and of about 11% for pitressin. The weights of the control frogs did not vary more than 1.5% from the initial weights (Fig. 1).

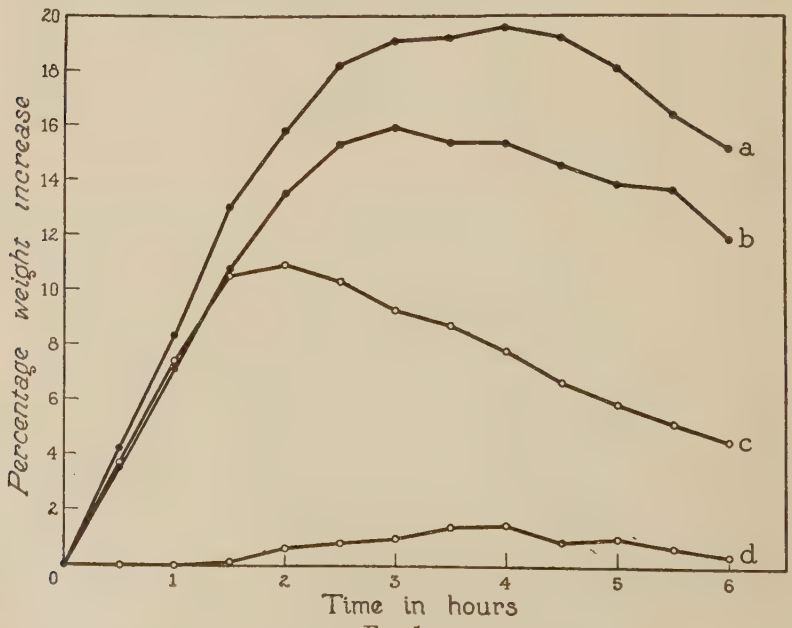

FIG. 1.

The influence of injections of 0.1 cc. per 10 gm. of body weight of preparations of the posterior lobe of the pituitary gland on the weights of frogs. *a*, pitocin, *b*, pituitrin, *c*, pitressin, and *d*, controls. Each line represents the average values for twelve different frogs.

The results of these experiments led us to inquire whether the absorbed water was lodged in the subcutaneous spaces or whether it was partially taken up by the body tissues. To investigate this problem, drainage of the subcutaneous spaces along the sides, back, and legs of 3 frogs was effected by opening the skin in those regions. These frogs then received intraperitoneal injections of pituitrin 0.1 cc. per 10 gm. of body weight; at the same time 3 intact frogs received similar injections, and the changes in weight were followed as in previous experiments. Both groups of frogs increased appreciably in weight, although in an equal period of time the average weight of frogs with intact skins exceeded that of frogs with opened skins by more than 5%. At the end of 3½ hours, when the increase in weight had not yet reached its maximal point, the skins of the intact frogs were opened in the same manner as had been done with those in the other group, and all the frogs were then reweighed. It is interesting to note that almost immediately following this procedure the weights of frogs of the 2 groups became almost identical. This sudden loss in weight following subcutaneous drainage indicates that a part of the water taken up is held in the subcutaneous spaces. Since there was a very decided increase in the weights of the frogs whose skins were opened at the beginning, and since the average weight of frogs in the second group after incision approximated that of frogs in the first group so closely, it is quite evident that a considerable amount of water is absorbed by the body tissues.

Since preparations of the posterior lobe of the pituitary gland were found to have so marked an influence on the water-absorption of frogs, we wondered what influence they might have on the rate of loss of water. To answer this question, 6 normal frogs were removed from the water and were placed in separate, large-meshed wire cages, to prevent huddling and to insure more uniform exposure of body surface. After control weights had been obtained, 3 of the frogs received injections of pitocin, 0.1 cc. per 10 gm. body weight, and the remaining 3 were kept as controls. All were then exposed to the breeze of an electric fan for about 2 hours and 30 minutes. All the frogs lost appreciably in weight, but those which had received injections of pitocin gave evidence of a greater loss of weight than those kept as controls. These observations would indicate that pitocin affects the frog in such a way that fluid is lost from the body at a more rapid rate than normally. Reddening of the ventral surface of the hind legs, which is seen in frogs after severe loss of water, occurred among those frogs that had been given in-

jections an appreciable time before it did among those which were not given the injections.

Summary and Conclusions. Preparations of the posterior lobe of the pituitary gland cause a marked increase in the weight of frogs which is found to be due to increased absorption of water. A comparison of the effects of pitocin, pituitrin, and of pitressin in producing an increased absorption of water indicates that pitocin is the most effective of the 3 in this regard. With the doses used, pitocin caused an average increase in weight of about 19%, whereas pitressin caused an increase of only 11%. The effects of pituitrin come practically midway between the other 2. About 5% of the excess water taken in through the skin may be held in the subcutaneous spaces along the sides, back, and legs. The rate of loss of water when frogs are removed from the water is definitely increased by injections of pitocin.

7710 P

Utilization of Calcium Salts by Children.

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Children 4-12 years of age have been given calcium salts in amounts such that the calcium content was equivalent to that in a pint or quart of milk. The retentions of calcium and phosphorus have been determined and compared with the retentions from the equivalent quantities of milk. The calcium and phosphorus retentions of children from 1 to 4 years of age have been determined when a quart of milk was given as the chief source of calcium, and when a calcium salt was substituted for one pint of the milk. A few studies were made wherein the salt was substituted for all of the milk. The protein intake of each diet was kept approximately constant during the salt and milk periods. The salts used were calcium lactate, carbonate, gluconate, and the di- and tri-phosphates. The latter was given either as the salt or in the form of a purified bone meal. No difference was observed in the relative retentions from the two sources. The results are summarized in Table I.

In general, the calcium and phosphorus retentions when the calcium phosphates were fed, were approximately equal to those from

TABLE I.
Utilization of Calcium Salts by Children.

Age Range yr.	No. of Exp.	Source of Ca	Aver. daily intake Ca gm.	Aver. daily intake P gm.	—Aver. daily retention— Ca gm.	—Aver. daily retention— P gm.	—Aver. daily retention— N gm.
1-4	34	milk 1 qt.	1.3	1.0	.021	.013	.040
	3	" 1 pt. + $CaHPO_4$	1.45	1.2	.024	.016	.120
	1	$CaHPO_4$	1.66	1.7	.026	.028	.150
	4	milk 1 pt. + Ca lactate	1.45	.8	.018	.011	.090
	1	$CaCO_3$	1.6	.32	.055	.014	.157
	3	milk 1 pt. + Ca lactate	2.1	.8	.034	.010	.099
	2	$CaHPO_4$	3.0	2.8	.043	.051	.236
4-7	19	milk	.7	.9	.007	.010	.073
	3	$CaHPO_4$	.75	.95	.008	.007	.036
	5	$CaHPO_4$*	.75	.90	.025	.018	.024
	6	$Ca_3(PO_4)_2$†	.45	.55	.004	.003	.038
	5	$Ca_3(PO_4)_2$†	.75	.75	.010	.008	.059
	3	Ca gluconate	.75	.47	—.003	—.009	.024
	23	milk	1.45	1.4	.014	.011	.042
	2	$CAHPO_4$	1.45	1.45	.010	.011	.054
	5	$CaHPO_4$*	1.45	1.40	.047*	.038*	.034
	1	$Ca_3(PO_4)_2$	1.1	.96	.013	.006	.029
	1	$Ca_3(PO_4)_2$*	2.1	1.4	.045*	.025*	.028
7-12	13	milk	.8	1.05	.007	.008	.108
	3	$CaHPO_4$	.8	.7	.008	.011	.128
	3	Ca gluconate	.87	.7	.007	.006	.094

*After periods of low Ca intake.
†$Ca_3(PO_4)_2$ as salt or purified bone meal.

equivalent quantities of milk. The very high retentions observed when these salts were fed after periods of low calcium intake indicate that all forms used are well absorbed by the average child of these ages. The retentions with the other calcium salts, the gluconate, lactate and the one study with calcium carbonate, are not so consistent as those obtained with the calcium phosphates. A greater individual variation was observed in the quantity of calcium and phosphorus retained by the different children. In general, it appeared that when the intake levels of calcium and phosphorus were approximately equal, the retentions of both these elements were good in relation to the intake, but that the greater the difference between the intake levels of calcium and phosphorus, the less satisfactory were the relative retentions of these elements. Calcium was well retained by all except two 6-year-old children given calcium gluconate. Phosphorus was well retained only when the intake was nearly equal to that of calcium.

In view of these findings, it is suggested that when calcium salts are given to children for purposes of retention, care must be observed to keep the calcium and phosphorus intakes approximately equal. For this reason, the calcium phosphates seem more de-

pendable as sources of calcium for the growing child than the other calcium salts studied.

7711 P

Effect of Cobalt Sulfate on Erythrocyte Count of the Splenectomized Albino Rat.*

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Prior to 1929 little was known of the biological behavior of cobalt. Waltner and Waltner[1] showed that inorganic salts of cobalt when fed or injected into normal albino rats produced a remarkable polycythemia. Orten, Underhill, Mugrage and Lewis[2] obtained similar results. The literature of cobalt polycythemia has now become quite extensive.

Our experiments were undertaken to determine if polycythemia can be produced by cobalt in splenectomized albino rats.

Orten, Underhill, Mugrage and Lewis[2] ashed the organs, finding the largest amounts of cobalt in the liver, pancreas and spleen and minute quantities in the bone marrow.

Since it is not possible to remove the liver or pancreas in recovery experiments, cobalt was tried on splenectomized rats, in view of the long disputed question of the hematopoietic spleen function.

Fourteen healthy, adult, white rats, mostly of the Wistar strain were used, 8 being splenectomized under sodium pentobarbital (40 mg./kg.) and morphine sulfate (10 mg./kg.) anesthesia. An incision 2.5 cm. long was made just below the left costal margin. The lieno-renal, gastrosplenic and splenic vessels were tied and the spleen removed aseptically; the animals then placed for an hour in oxygen 90%, carbon dioxide, 10%.

After 3 weeks, cobalt injections were started. Duplicate erythrocyte counts were made routinely before and after injections; at first, weekly; later, fortnightly, using Max Levy counting chambers and Yankee Certified mixing pipettes, and Hayem's diluting fluid. Two control splenectomized rats were used. The remaining 6 operated

* This research was aided by a grant from the Hendricks Research Fund.

1 Waltner, K., and Waltner, K., *Klin. Wochneschr.*, 1929, **8**, 313.

2 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 174.

animals and 6 unoperated animals received cobalt daily. Blood was taken from the projecting tail, the animal being confined in a closely fitting box, to avoid excitement. Animals' weights were charted at each count; and at the height of the polycythemia in the normal animals, specific gravity tests on all of the rats were made by the chloroform-benzene method without significant variations from the normal.

TABLE I.
Erythrocyte Counts on Various Groups.

Group	Count in millions/mm.^{3} before injection	Count in millions/mm.^{3} days after injection		Difference	Daily Dose in mg.
Normal plus cobalt	8.42	40	12.78	4.36	2
	7.65	40	12.20	4.55	2
	9.31	55	12.75	3.44	1
	10.03	55	13.01	2.98	1
	8.15	10	10.95	2.80	3
	8.35	10	11.08	2.73	3
Splenectomized plus cobalt	8.20	40	9.90	1.70	2
	7.85	40	8.24	0.39	2
	8.63	10	7.56	—1.07	3
	9.62	10	10.49	0.87	3
	8.45	55	4.14	—4.31	1
	9.45	55	2.63	—6.82	1
Splenectomized with no cobalt	8.71	40	9.64	0.93	2
	7.29	40	7.77	0.48	2

Results. Table I shows cobalt polycythemia graphically in 6 normal animals; beginning from the minimum daily dose (1 mg.) in about 3 weeks; from the maximum (3 mg.) after one week; average increase in erythrocytes being nearly 50%. Two treated operated animals showed extreme anemia probably due to *Bartonella muris* infection, common following splenectomy in the albino rat. Four treated, operated animals maintained normal counts as well as 2 untreated operated animals. Splenectomy in non-infected *(Bartonella muris)* animals produced no variations in blood count except for a slight anemia the second or third day after operation. All animals gained in weight during the experiment. Six weeks after injections were stopped previously polycythemic rats had normal erythrocyte counts.

7712 P

Shock Due to Freezing: I. Shift of Body Fluids and Associated Blood Concentration Changes.*

HENRY N. HARKINS. (Introduced by Edmund Andrews.)

From the Department of Surgery, The University of Chicago.

It has been shown by Blalock,[1] Underhill[2] and the writer[3] that in shock due to severe burns there is a loss of fluid from the blood stream into the burned tissues. It is considered by these authors that this loss of fluid is responsible for a large part of the shock resultant to burns. The amount of fluid shift into the tissues has been measured by burning one lateral half of an animal and then after careful sagittal bisection, comparing the weight of the burned and unburned sides;[1] by weighing the fluid expressed from the water-logged burned tissues;[2] and by burning one lateral half of an animal placed on a balanced apparatus and measuring the amount of displacement caused by the increase in weight of the burned side.[3]

Accompanying this loss of fluid there is a marked blood concentration and in shock in human beings resulting from severe burns, Underhill[4] found that hemoglobin readings as high as 209% indicate a marked blood concentration. Blalock found that in experimental burns the hemoglobin may rise to 130%. The writer[3] found that in experimental burns the hemoglobin may rise to as high as 162% (Sahli: 17 gm. per 100 cc. = 100%) and the hematocrit reading to 72.

It was thought that the severe general effects of freezing a portion of the body might be due to a similar leakage of fluid from the blood stream into the frozen or thawing tissues with a resultant blood concentration. Solid carbon dioxide was applied to about one-fourth of the body surface of 10 completely anesthetized dogs and left in place for about an hour, at the end of which time the underlying tissues were deeply frozen. In all but the ninth experiment, in which the shift of body fluid was not determined, all of the freezing was done on portions of one lateral half of the animal.

* Work done in part under a grant from the Douglas Smith Foundation.

1 Blalock, A., *Arch. Surg.*, 1931, **22**, 610.

2 Underhill, F. P., Fisk, M. E., and Kapsinow, R., *Am. J. Physiol.*, 1930, **95**, 325.

3 Harkins, H. N., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 994; *Arch. Surg.*, in press.

4 Underhill, F. P., Carrington, G. L., Kapsinow, R., and Pack, G. T., *Arch. Int. Med.*, 1923, **32**, 31.

Frequent blood pressure readings, hemoglobin percentages and hematocrit readings were determined. The blood pressure was obtained by placing in the carotid artery a cannula which was connected to a mercury manometer. Hemoglobin determinations were made by the Sahli method and hematocrit readings with the Van Allen hematocrit.

Results. The initial blood pressure averaged 148 and the final blood pressure 81 mm. of mercury. This amount of blood pressure lowering may be considered arbitrarily as within the limits of so-called surgical shock. After a period averaging 21 hours from the time of freezing, 4 of the dogs were dead and the other 6 were killed. Nine of the animals were then carefully bisected by the method previously described[3] and a comparison made of the weights of the frozen and unfrozen sides. The average difference amounted to 2.55% of the total body weight. Incision into the tissues that had been frozen revealed sufficient plasma-like fluid to account for the difference in weight. This amount of plasma, if lost from the blood stream, is sufficient to account for a large part of the shock present in these animals.[1] In 8 burn experiments previously reported,[3] the amount of fluid shift at death was 2.2% of the total body weight.

The blood concentration changes began almost immediately after the freezing and were present long before the blood pressure had fallen markedly. It is seen from Table I that in all animals there

TABLE I.

Shift of body fluid and associated blood concentration changes in shock due to freezing. The duration of the experiment indicates the time of death of the animal after freezing. The animals in experiments 1, 2, 3, 4, 8, and 9 were killed immediately following the last blood pressure reading; the animals in the other experiments died some time following the last reading. The hemoglobin and hematocrit figures recorded after freezing indicate the highest readings in each experiment. The lateral shift of body fluids is determined by the bisection method.

Exp.	Wt. of dog, kg.	Duration of exp. hr.	Blood pressure mm. of Hg. Initial	Blood pressure mm. of Hg. Final	Hemoglobin, % Control	Hemoglobin, % After freezing	Hematocrit Control	Hematocrit After freezing	Shift of body fluid as % of body wt.
1	12.2	26	160	114	76	131	36	63	5.14
2	13.8	28	138	92	75	136	37	59	0.15
3	16.0	26	164	96	102	113	48	58	2.44
4	7.3	17	156	94	93	104	46	53	1.51
5	8.9	20	118	42	104	160	48	74	4.05
6	11.1	25	168	70	97	128	46	63	2.92
7	11.0	17	159	38	123	159	61	74	2.77
8	8.3	13	120	64	85	116	42	61	1.35
9	23.5	16	158	76	96	163	50	70	—
10	7.1	18	142	120	109	168	54	80	2.59
Aver.	11.9	21	148	81	96	134	47	66	2.55

was an increase in hemoglobin percentage and hematocrit reading. The average hemoglobin percentage before freezing was 96 and after freezing was 134. The average hematocrit reading before freezing was 47 and after freezing 66. The figures obtained in experiment 10 are higher than in any readings obtained in shock due to burns, except by Underhill in human beings. Control experiments showed no marked blood concentration and no fluid shift.[3]

7713 P

Shock Due to Freezing: II. Composition of Edema Fluid.*

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From the Department of Surgery, The University of Chicago.

The composition of the fluid that escapes into the subcutaneous tissues after burns has been determined by Beard and Blalock.[1] These authors found that in general the chloride content of the fluid was higher than that in blood plasma, the concentration of sugar and non-protein nitrogen was approximately the same in the 2 media and the protein content of the subcutaneous fluid was about 20% lower than that of the blood plasma. Underhill and Fiske[2] made similar comparisons between the tissue fluid after burns and the blood serum. Their results agree in general with those of Beard and Blalock, except that they found the non-protein nitrogen content considerably higher in the edema fluid than in the blood serum.

The results of these authors indicate that the fluid that escapes into the subcutaneous tissues after burns very closely resembles blood plasma. The escape of a plasma-like fluid undoubtedly produces more serious consequences than the escape of a simpler solution. Hence, it was considered of importance to determine whether the fluid that escapes into the subcutaneous tissues in large amounts after freezing is of a plasma-like nature similar to that following burns. Nine completely anesthetized dogs in which shock was produced by freezing portions of the body with solid carbon dioxide were dissected after death and large amounts of edematous subcu-

* Work done in part under a grant from the Douglas Smith Foundation.

1 Beard, J. W., and Blalock, A., *Arch. Surg.*, 1931, **22**, 617.

2 Underhill, F. P., and Fiske, M. E., *Am. J. Physiol.*, 1930, **95**, 330.

taneous tissue found. By making multiple incisions in this tissue, sufficient fluid drained out for analysis. This fluid was only slightly tinged with blood, but clotted if no anticoagulant was added. A similar amount of anticoagulant was added to the edema fluid and to a sample of blood obtained from the carotid artery. Analyses were made of the fluid and of the blood plasma. The sugar was determined by the Folin '29 modification method on a sulfate-tungstate filtrate; the sodium chloride by the Eisenman open-Carius method; the protein by the Koch-McMeekin micro-Kjeldahl method; and the non-protein nitrogen by the same method on a Folin-Wu filtrate.

TABLE I.

A comparison of the concentration of certain substances in blood plasma and in the fluid that escapes into the subcutaneous tissues after freezing. The value for the sugar in the fluid in experiment 7 was too low to read.

Exp.	Sugar Mg. per 100 cc.		NaCl Mg. per 100 cc.		Non-protein Nitrogen Mg. per 100 cc.		Protein Gm. per 100 cc.	
	Plasma	Fluid	Plasma	Fluid	Plasma	Fluid	Plasma	Fluid
1	—	95.2	—	711.4	—	38.5	—	3.9
2	132.5	101.0	—	665.7	40.0	35.3	4.4	2.9
3	—	81.0	—	758.2	—	36.8	—	3.2
4	44.1	98.0	673.9	688.0	56.8	50.8	3.3	3.3
5	90.1	13.4	654.0	665.7	95.4	76.3	3.7	3.8
6	84.0	18.9	700.8	703.2	27.0	33.3	4.8	3.6
7	94.2	—	709.0	703.2	45.5	60.6	5.1	3.4
8	177.8	111.1	663.4	690.3	60.3	44.2	3.4	4.7
9	—	82.1	655.2	686.8	—	73.2	4.6	3.7
Aver.	103.8	66.7	676.1	696.9	54.2	49.9	4.2	3.6

The results of these analyses are shown in Table I. The sugar concentration is in general lower in the fluid than in the blood plasma. The extremely low values in several instances are of interest. The chloride concentration is approximately the same in the blood plasma and tissue fluid. The non-protein nitrogen and protein are somewhat higher in the plasma than in the tissue fluid.

Conclusion. The composition of the edema fluid that escapes into the subcutaneous tissues after freezing is quite similar to blood plasma. This indicates that the loss of large amounts of such plasma-like fluid from the blood stream might account, in part at least, for the shock resultant to the freezing.

7714 P

Function of De-afferented Amphibian Limbs.

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In analyzing the so-called resonance phenomena of nervous control, discovered by the author,[1] the necessity arose to reinvestigate the problem of the significance of afferent excitations for the establishment and persistence of motor functions in Amphibia. In a number of toads and axolotls, limbs were de-afferented by removing all the spinal ganglia and attached dorsal roots of the limb segments (fore limb, hind limb or both), either on both sides or on one side only. In contrast to most of the earlier experiments, the operation was performed without opening the vertebral canal so that injury or degenerative damage to the motor roots could be strictly avoided. In one series the sympathetic system has been preserved, in another series it was removed within the de-afferented area, including ganglia and rami communicantes.

A. *Experiments with toads.* Twenty-eight animals were operated (in ether anesthesia) and examined as to their motor functions from immediately after the operation to 22 days later.

I. *Motility.* After bilateral de-afferentation of the hind legs (17 cases) no essential change from normal could be noticed in the performance of locomotor functions: jumping and walking on rough ground are well-aimed, well-coordinated and vigorous. The reflexes of righting, turning and wiping are performed in the normal way. The resting posture is normal, except for a certain lack of contact between the plantar surface of the foot and the ground. It is evident, therefore, that after de-afferentation the motor functions of the affected limbs persist without significant alterations. This is strikingly borne out by unilateral de-afferentation, no asymmetry in jumping, righting, etc., appearing in such animals.

No difference could be found in the motor functions between animals possessing (14 cases) or deprived of (11 cases) the sympathetic chain.

De-afferentation of the fore limbs alone was effected in one case only. The locomotion of the animal was not markedly impaired; wiping reflexes of the arms could be obtained but the hind limbs were used preferentially. A certain hypertonicity of the flexors and pronators was characteristic of the resting posture.

[1] Compare the recent review by DeSilva, H. R., and Ellis, W. D., *J. Gen. Psychol.*, 1934, **11**, 145.

Serious disturbances resulted from the combined de-afferentation of both the fore and the hind limbs (2 cases). Such animals were extremely apathetic and hardly ever moved "spontaneously". If, however, they could be induced to move, their jumping, walking, righting and turning were coordinated.

II. *Tonus.* Neither the view that the maintenance of the tonic posture requires proprioceptive control nor the assumption that it is due to sympathetic innervation could be substantiated by these experiments on the toad. In order to test the tonic (postural) phenomena, the animals with de-afferentated hind limbs were suspended vertically, head upward. Hanging freely, they kept their hind legs completely adducted and flexed. This posture could be maintained for several minutes (longest observed time, 9 minutes), in some specimens without any dropping of the legs at all, in some others with a gradual drop to a half-relaxed position; great individual differences were observed between more "motile" and more "tonic" individuals. The fact that the de-afferentation does not visibly impair the ability to assume and maintain tonic postures against gravity is most strikingly demonstrated after unilateral de-afferentation. In the suspension tests of such animals no marked asymmetry of posture can be discerned between the de-afferented limb and the normal limb of the opposite side; when the limbs drop, they do so simultaneously, to the same extent. Asymmetry due to a loss of tonus in the de-afferented limb is, however, observed whenever the motor supply has been damaged in addition to the de-afferentation. If motor damage has been avoided, the asymmetry described for the first time by Brondgeest[2] for the frog could never be obtained on the toad with intact brain. We must conclude, therefore, that proprioceptive control is at least not indispensable in the production and maintenance of muscular tonus in Amphibia.

The tonus of de-afferented limbs is "plastic" in one direction: when the limb with tonic flexion is passively extended, it remains in the new extended position without tending to return to the old posture; on the other hand, one invariably fails in an attempt to make the limb resume its flexed position by passively flexing it.

The sympathetic nervous system proved to be entirely unessential for the maintenance of tonus. Animals bilaterally de-afferented and unilaterally sympathectomized assumed perfectly symmetrical postures in the suspension tests. Possibly the sympathectomized leg showed fatigue slightly earlier in some cases, but certainly not as a rule.

[2] Brondgeest, P. J., *Arch. f. Anat., Physiol. and wiss. Med.*, 1860, 703.

B. *Axolotl.* In axolotls of about 5 to 7 cm. in length the spinal ganglia and dorsal roots were removed in the arm segments (2nd, 3rd, 4th, and 5th) on one side. Owing to motor injury, 2 out of the 10 operated animals showed partial motor paralysis. In the remaining 8 cases, however, observed from the operation up to 4 weeks afterwards, the locomotor functions of the de-afferented limbs were found to be perfectly normal and undisturbed. In walking the limbs were used in normal coordination. In a few cases there seemed to be a slight hypertonicity of the extensor and adductor muscles in the resting position, which did not, however, even in the most extreme cases, impede the normal motor activity of the limb.

7715 C

Effect of Irradiated Ergosterol and Calcium Lactate on Calcification of Trichina Cysts.*

W. W. WANTLAND. (Introduced by F. T. Jung.)

From the Department of Zoology, Northwestern University.

Workers in the field of experimental parasitology have repeatedly shown that in the normal course of infection with *Trichinella spiralis,* cysts begin to form around the coiled larvae in the muscle fibers in 4 to 6 weeks following infection. These cysts, at first extremely delicate, gradually become more conspicuous and after about 7 or 8 months there begins a deposit of calcareous material, resulting finally in calcification of the entire cyst. Complete calcification of the cyst and the contained larvae seldom occurs normally in less than one year and in many instances the calcification process is much slower.

It seems quite probable that the more general symptoms of trichiniasis, muscular pains, fever, eosinophilia, etc., are, in part at least, due to toxic products formed by the breaking down of large amounts of muscle tissue together with waste products of the larvae. Thus a continuous inoculation of the infected host with toxic proteins occurs. When cyst formation, the protective mechanism of the body for walling off the parasite, has progressed sufficiently, production of poisonous products and the effects of mechanical irritation by the larvae tend to be inhibited.

* This series of experiments was performed at Northwestern University, Department of Zoology, under the direction of Dr. Franklin D. Barker.

It is significant that the majority of deaths from trichiniasis occur 4 to 6 weeks after infection, during that period immediately preceding, or during the earlier stages of, cyst formation. It would seem then that if cyst formation and subsequent calcification could be hastened this would shorten the critical period in trichiniasis and more quickly terminate the disease.

It has been shown (Zucker and Matzner[1]) that irradiated ergosterol through the action of the active vitamin D, increases the absorption of calcium from the intestine in rats and (Harris and Moore[2]) produces a heavy calcification in heart muscle, kidneys and other organs in rabbits.

Eight white rabbits, in 2 groups of 4 each, were fed approximately 5,000 Trichinella larvae, previously digested out in artificial gastric juice.

In the first group 2 animals were given irradiated ergosterol and calcium lactate by mouth and 2 animals were kept as controls (see Table I, Series A).

An examination of Table I shows that the feeding of irradiated ergosterol and calcium lactate, in the amounts indicated, resulted in a marked acceleration of the calcification process. Marked calcification occurred within 2 months and 28 days equal in degree to that which normally requires 8 to 12 months.

The physical condition and appetite of the 2 treated rabbits were considerably better than that of the untreated controls.

In the second group, 3 rabbits were given irradiated ergosterol† and calcium lactate by mouth and one rabbit was kept as a control (see Table I, Series B). Rabbit No. 26 died approximately 4 weeks following infection. Slight calcification of cysts was noticed upon microscopic examination of the diaphragm. Rabbit No. 27 died near the end of the sixth week following infection. Marked calcification of cysts was observed in the diaphragm and various other striated muscles. Rabbit No. 25 and Rabbit No. 29, the control, were killed on this same date. Marked calcification of cysts in the diaphragm of No. 25 was evident but no calcification of cysts was visible in No. 29.

The rate of calcification varies directly with the amount of irradiated ergosterol and calcium lactate given and the length of time which the rabbits were under treatment. The differences in the

[1] Zucker, T. F., and Matzner, M. J., PROC. SOC. EXP. BIOL. AND MED., 1924, **21**, 186.

[2] Harris, L. J., and Moore, T., *Biochem. J.*, 1929, **23**, 261.

† Mead's Irradiated Viosterol in oil 10,000 International (also U. S. Pharmacopeia) units of vitamin D per gm. One Steenbock unit equals 2.7 U. S. P. units.

TABLE I.

	Series A				Series B			
	Controls		Treated		Control	Treated		
	R8	R9	R11	R12	R29	R25	R26	R27
Approximate No. larvae fed	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000
Date of infection	12/5/33	12/5/33	12/5/33	12/5/33	3/17/34	3/17/34	3/17/34	3/17/34
Treatment begun			2/5/34	2/5/34		3/20/34	3/20/34	3/20/34
Dosage per day ergosterol and calcium lactate	None	None	16 drops 0.5-1 gm.	20 drops 0.5-1 gm.	None	80 drops 1 gm.	100 drops 1.5 gm.	150 drops 2 gm.
Autopsy	3/4/34 (Killed)	3/3/34 (Killed)	3/3/34 (Killed)	3/3/34 (Killed)	4/27/34 (Killed)	4/27/34 (Killed)	4/16/34 (Died)	4/27/34 (Died)
Calcification of cysts	None	None	Marked	Marked	None	Marked	Slight	Marked

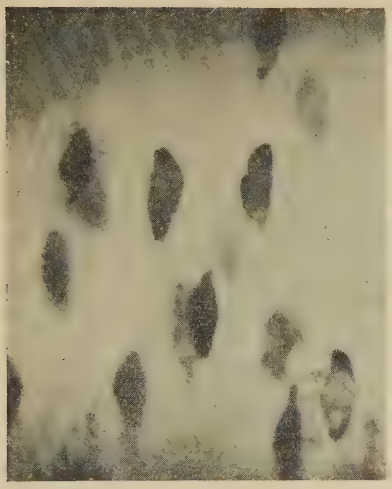

FIG. 1.

FIG. 2.

FIG. 1. Photomicrograph of fresh diaphragm of Rabbit No. 27. 32.5 ×. Animal received 150 drops of irradiated ergosterol plus 2.0 gm. calcium lactate every day.

FIG. 2. Another region, Rabbit No. 27. 85 ×.

degree of calcification are shown in the photomicrographs of the fresh diaphragms.

That too large an amount of ergosterol may be given is indicated by the death of Rabbit No. 27. This animal had presumably passed through the more dangerous period of the disease but suddenly near the end of the sixth week, exhibited extreme muscular weakness, dyspnea and paroxysmal tachycardia. It is probable that large doses of activated ergosterol lead to a hypercalcemia and subsequent calcium rigor.

A test was made on another rabbit (No. 24) infected on the same date as the above group in Series B. This animal had been receiving 60 drops of ergosterol plus 0.5 gm. of calcium lactate every other day. On April 27 the dosage was increased to 180 drops of ergosterol plus 2.0 gm. of calcium lactate per day. On May 1 the animal refused most of its food and on May 2 exhibited extreme weakness, increased heart rate and dyspnea. The animal was killed and marked calcification of cysts was noted.

Five other white rabbits were infected on the same date and with approximately the same number of larvae as the animals in Series

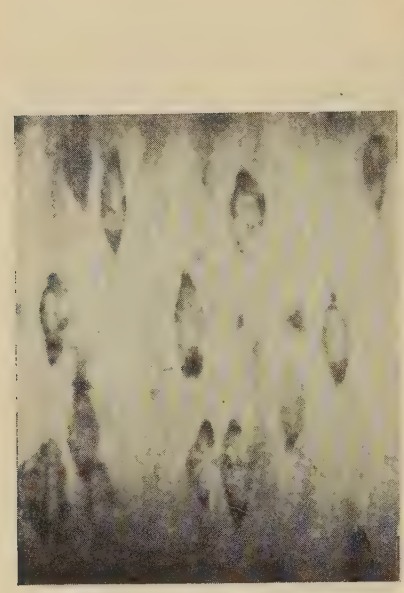

FIG. 3.

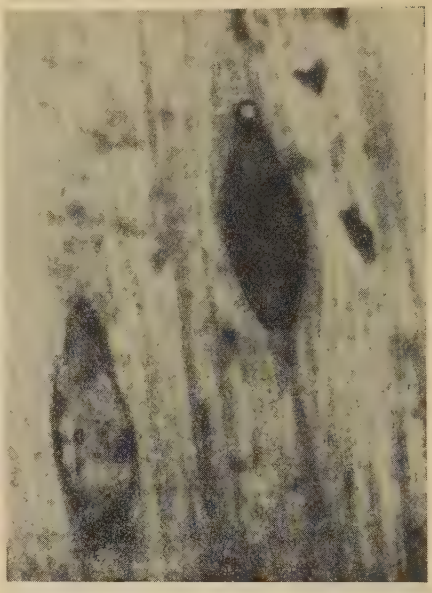

FIG. 4.

FIG. 3. Photomicrograph of fresh diaphragm of Rabbit No. 25. 32.5 ×. Animal received 80 drops of irradiated ergosterol plus 1.0 gm. calcium lactate every day.

FIG. 4. Another region, Rabbit No. 25. 85×.

B. Two of these animals (R-22 and R-23) received respectively 30 drops of ergosterol plus 0.5 gm. of calcium lactate and 40 drops of ergosterol plus 0.5 gm. of calcium lactate every other day since the third day following infection. The remaining 3 were control animals.

Muscle tissue from rabbit No. 23 showed marked calcification of cysts 2 months and 13 days from the date of infection. Calcification of cysts was observed in Rabbit No. 22, but in a lesser degree, 3 months and 10 days from the date of infection. These 2 treated animals did not show any symptoms of trichiniasis. They remained active, increased in weight and were in excellent condition at the time of killing.

The 3 control animals on the other hand exhibited symptoms in varying degrees. Dyspnea appeared, extreme muscular weakness and loss of appetite. In all 3 cases, however, the symptoms subsided (except for general weakened condition) during the sixth week of infection and the animals lived until they were killed 3 months and 10 days from the date of infection. No calcification of cysts had taken place in these control animals.

Six rabbits infected with from 4,000 to 5,000 larvae and treated

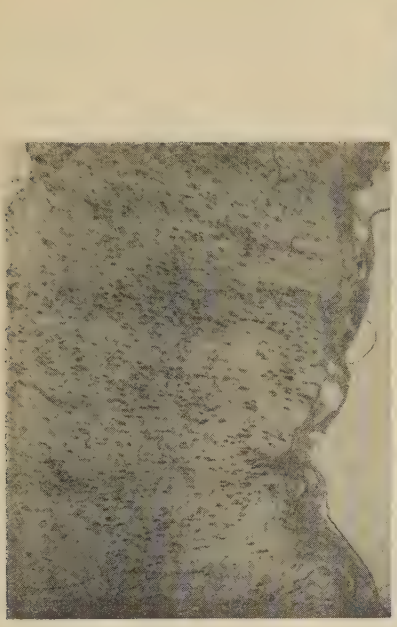

FIG. 5.

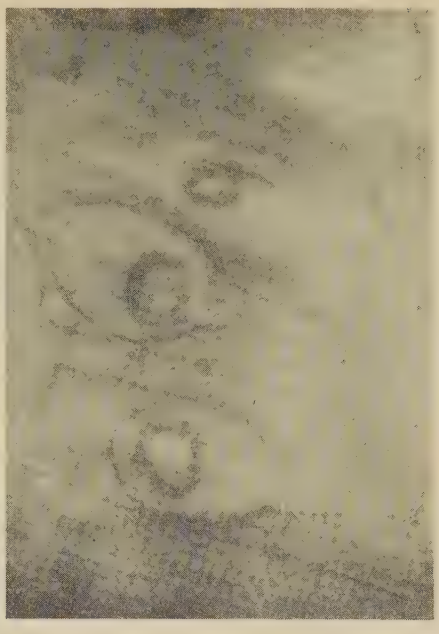

FIG. 6.

FIG. 5. Camera view of region of diaphragm, Rabbit No. 27. 1.5 ×. Small dark bodies are cysts.

FIG. 6. Photomicrograph of fresh diaphragm of Rabbit No. 29 (control animal). 85 ×. Animal infected same date as Nos. 25 and 27. No treatment.

with from 16 to 20 drops of irradiated ergosterol plus from 0.5 gm. to 1.0 gm. of calcium lactate every other day showed no calcification of cysts at autopsy (4 weeks after infection). In 2 other infected animals, however, that had received respectively 50 drops of ergosterol plus 1.5 gm. calcium lactate and 60 drops of ergosterol plus 2.0 gm. calcium lactate every other day, slight calcification was observed during the fourth week of infection. At the end of the fourth week the poles of the cysts show medium to marked calcification in animals that have received between 30 and 60 drops of irradiated ergosterol plus from 0.5 gm. to 2.0 gm. of calcium lactate every other day. The onset of the calcification process in treated animals evidently takes place during the early part of the fourth week following infection.

The optimum dosage of activated ergosterol as evidenced from the above experiments is from 30 to 60 drops every other day. This amount apparently has no deleterious effects yet markedly accelerates the calcification of trichina cysts in white rabbits. The statement that in the normal course of infection cysts begin to show

signs of calcification only after 7 or 8 months from the date of infection has been set forth by various observers in the field of parasitology and is in agreement with the experiments performed in this laboratory. The results of the experiments here reported are in striking contrast to the normal course of trichiniasis as shown by the marked calcification obtained in less than 6 weeks in treated animals by the administration of irradiated ergosterol and calcium lactate.

The treatment of trichinized rabbits with irradiated ergosterol apparently has a definite therapeutic value. It still remains to be tested in human cases of trichiniasis.

7716 P

Fibrinolytic Streptococci from Lower Animals.*

R. R. MADISON. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

In addition to hemolytic streptococci specifically lytic for human fibrin there are at least 2 apparently distinct fibrinolytic strains of *S. hemolyticus* (or strains intermediary between *S. hemolyticus* and

TABLE I.

Lysis of Lower Animal Fibrins by *S. hemolyticus*.

24-hour veal-infusion broth cultures of *S. hemolyticus* tested against veterinary fibrins by the serum-free fibrin-clot technic of Tillett and Garner.[1]

++++ represents complete liquefaction of the fibrinogen-thrombin complex within 10 minutes; +++, 30 minutes; ++, 1 hour; and +, 2 to 3 hours.

+++ and ++++ fibrinolytic strains are + and ++ thrombolytic, by the plasma-clot technic.

Origin of strain	Fibrin				
	Horse	Hog	Cow	Rabbit	Man
Horse, "Strangles" (10 strains)	+	0	0	0	0
" "	++	0	0	0	0
Colt, "Navel ill"	+++	0	0	0	0
Rabbit, "Pneumonia"	+	0	0	0	0
Man, "Prostate abscess"	+	0	0	0	+
" "Knee "	+	0	0	0	++++
Hog, "Septicemia" (2 strains)	0	++++	0	0	+
" "Mixed infection"	0	+	0	0	0
Control (Autolytic test)	0	0	0	0	0

* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

S. viridans), which are apparently equally specific for the fibrins of certain domestic animals. A summary of our streptococcus strains which dissolve fibrins from lower animals is given in Table I.

7717 C

Enzyme-Concentration Method of Titrating Bacterial Fibrinolysins.*

R. R. MADISON. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

Since but 17% of all local strains of *Streptococcus hemolyticus* originally isolated from superficial infections of man are demonstrably thrombolytic[1] by the Tillett-Garner plasma-clot technic,[2] we have retitrated these strains by their more delicate serum-free fibrin-clot method. This retitration has raised the percentage of demonstrably fibrinolytic strains to 25%.

It is of basic interest to determine whether or not the remaining 75% have or have not a demonstrable antihuman fibrinolytic capacity. To determine this we have again retitrated all local strains, using a modification of the Tillett-Garner enzyme-concentration method.

Since at least 75% of the fibrinolysin in a 24-hour broth culture of *S. hemolyticus* is usually lost as a result of filtration, enzyme-concentrates were prepared from unfiltered broth cultures. To prepare such a concentrate, 20 cc. unfiltered 24-hour veal-infusion broth culture is added to 60 cc. 96% alcohol, both culture and alcohol being ice-cold at the time of mixing. The resulting mixture is allowed to stand at refrigerator temperature for at least an hour. The precipitate is then collected by centrifugation, and rapidly dried in a vacuum desiccator.

To make the fibrinolytic test, the crude precipitate is suspended in 1 cc. buffered salt-solution and freed from undissolved residue by centrifugation. The resulting centrifugate usually has a fibrinolytic titer at least 20 times that of the original broth-culture, which is generally equivalent to at least 80 times that of the broth-filtrate.

* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1018.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

Retitration of 123 local strains of *S. hemolyticus* originally isolated from superficial infections of human beings has raised the percentage of recognizable fibrinolytic strains to 35%. About one-eighth of all apparently non-fibrinolytic strains of *S. hemolyticus* from infections in man, therefore, have a demonstrable fibrinolytic potential, recognizable by the 20-fold enzyme-concentration technic.

By the same enzyme-concentration technique the percentage of strains of *S. hemolyticus* from infections of lower animals positively lytic for human fibrin has been raised from 7% to 22%. None of the 40 local strains of *S. viridans,* however, has shown a demonstrable anti-human fibrinolytic capacity.

7718 C

A Modified Method for the Estimation of Tryptophane.*

T. TOMIYAMA AND S. SHIGEMATSU. (Introduced by Carl L. A. Schmidt.)

From the Imperial Fisheries Institute, Tokyo, Japan.

This paper deals with a modification of May and Rose's[1] method for the determination of tryptophane. In their method the protein is dissolved in HCl which contains Ehrlich's reagent, incubated at 35° for 24 hours, and then allowed to stand for an additional 40 hours at room temperature. The blue color which is thus produced is matched against a similar color obtained by treating casein in an identical manner.

In this procedure such factors as the particular preparation of casein used for the standard and the room temperature may be uncontrolled variables. May and Rose assumed the tryptophane content of casein to be 1.5%. It is based on the data of Hopkins and Cole.[2] Other values have been reported.[3] The uncertainty as to the tryptophane content of casein makes this protein of questionable value as a standard.

* The authors wish to acknowledge their indebtedness to Professor Y. Okuda of Kyushu Imperial University for advice and suggestions during the experiments, and to Professor C. L. A. Schmidt of the University of California for his kindness in reading through the manuscript.

[1] May, C. E., and Rose, E. R., *J. Biol. Chem.*, 1922, **54**, 213.

[2] Hopkins, F. G., and Cole, S. W., *J. Physiol.*, 1902, **27**, 418.

[3] Herzfeld, E., *Biochem. Z.*, 1913, **56**, 258, 0.51%; Thomas,P., *Ann. Inst. Past.*, 1920, **34**, 701, 1.7-1.8%; Fürth, O., and Nobel, E., *Biochem. Z.*, 1920, **109**, 103, 2.02%; Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627, 1.4%; Holm, G. E., and Greenbank, G. R., *J. Am. Chem. Soc.*, 1923, **45**, 1788, 2.24%.

In the present work the attempt was made to use tryptophane for the production of the standard color. It was found that the development of the color does not proceed at the same rate when casein and tryptophane are treated with the reagent. Tryptophane reacts more slowly. The color given by tryptophane is not identical with that obtained when casein is used. At 45° both casein and tryptophane react more rapidly than at either 37° or 30°; however, at 45° both substances show only 60-80% of the maximum color intensity of that which was obtained at either of the 2 lower temperatures. Apparently considerable destruction of tryptophane takes place at 45°. At 30° the color is fairly stable and continues so for a period of 48 hours after reaching the maximum intensity. The present data are not entirely in accord with those reported by Holm and Greenbank.[4] Using the color of the copper ammonium complex as a standard, they found that the same maximum color intensity was obtained irrespective of whether tryptophane was treated with Ehrlich's reagent at 25° or 45°. The maximum color intensity in their experiments appeared in 8 days at 45°, in 12 days at 37°, and in 20 days at 25°. In the present experiments the maximum color was obtained in about 80 hours at 37°, and in 120 hours at 30°.

Herzfeld[5] has shown that the color of the copper ammonium complex salt is similar to that obtained when tryptophane is treated with the Ehrlich reagent. An objection to the use of the former color standard are the fumes of ammonium chloride which are formed when the protein or tryptophane which is treated with the hydrochloric acid-containing reagent is brought near the ammonia-containing copper reagent. In the present experiments it was found that the color produced by reduced phosphomolybdate which is made use of in the determination of phosphorus was quite similar to the color of the condensation product formed when tryptophane is treated with the Ehrlich reagent. For the estimation of tryptophane the blue color given by a standard phosphate solution prepared essentially according to Lohmann and Jendrassik's[6] modification of Fiske and Subbarow's method was adopted as a color standard. The preparation of the phosphate standard was carried out as follows:

1. Molybdate solution. 0.2% solution of ammonium molybdate in 5 N sulfuric acid.

2. Eikonogen solution. 0.5 gm. of Eikonogen (aminonaphthol

[4] Holm, G. E., and Greenbank, G. R., *J. Am. Chem. Soc.*, 1923, **45**, 1788.

[5] Herzfeld, E., *Biochem. Z.*, 1913, **56**, 258.

[6] Lohmann, K., and Jendrassik, L., *Biochem. Z.*, 1926, **178**, 419.

sulfonic acid) is dissolved in a mixture of 195 cc. of 15% $NaHSO_3$ solution and 5 cc. of 20% Na_2SO_3 solution.

3. Standard phosphate solution. 0.4394 gm. of KH_2PO_4 is dissolved in a liter of water, *i. e.*, 1 cc. is equivalent to 0.1 mg. P.

The standard color is prepared by pipetting 10 cc. of the standard phosphate solution, 5 cc. of the ammonium molybdate solution, and 1 cc. of the Eikonogen solution into a 25 cc. volumetric flask, the mixture is warmed in a water bath at 37° for 5 minutes, then cooled to room temperature with tap water, and brought to volume by the addition of distilled water. This solution is diluted 10 times and used as the color standard. It can be accurately reproduced and does not change appreciably over a 24-hour period. The color of the phosphate mixture is just equivalent to the color of a mixture which contained 0.860 mg. of tryptophane dissolved in 10 cc. of distilled water to which was added 2 cc. of 0.2% NaOH solution, 90 cc. of 19% HCl, and 1 cc. of Ehrlich's reagent, and incubated at 30° for 5-6 days, *i. e.*, the period which gave the maximum color intensity. The Ehrlich reagent used was a 5% solution of p-dimethylaminobenzaldehyde in 10% H_2SO_4. In estimating the tryptophane content of casein, about 50 mg. of the protein were brought into solution by warming at about 50° with 2 cc. of 0.2% NaOH.[7] After cooling, 100 cc. of 19% HCl and 1 cc. of the reagent were added and the solution was incubated at 30° until it gave the maximum color intensity.

The tryptophane content of Merck's Hammarsten casein was

TABLE I.
Course of Development of the Tryptophane Color in Various Kinds of Proteins when the Inorganic Color Standard Was Set at 20 on the Colorimeter.

Protein	Days of Incubation 3	4	5	6	8	10	12	Tryptophane content %	Folin and Ciocalteau[8] Values
Casein	29.7	23.8	21.6	21.4	21.5	21.6	25.0	1.7	1.4
Edestin	28.6	25.6	23.2	23.4	22.2	22.4	23.9	1.5	1.5
Egg Albumin	31.8	28.4	23.7	23.3	21.8	21.0	20.9	1.3	1.3
Protein of									
Soy Bean*	54.6	36.6	31.5	32.7	30.7	30.4	33.6	1.3	
Silk-worm Pupa*	56.8	33.1	26.8	25.6	23.6	22.1	22.6	1.5	
Muscle Protein of									
Bonito	63.8	46.4	38.8	37.4	33.7	31.0	30.0	1.2	
Whale*	54.2	38.4	33.3	33.5	31.3	28.9	38.1	1.2	
Sardine*	62.8	41.0	34.5	33.8	31.1	29.7	29.4	1.4	
Chicken	50.6	44.6	37.2	35.4	33.3	31.1	30.6	1.1	

*The preparation of these mixed proteins is described in *J. Biochem.* (Japan), 1934, **19**, 345.

[7] Matsuyama, Y., and Mori, T., *Nippon Kagaku Kaishi*, 1923, **44**, 377.

found to be 1.7%. This is somewhat higher than the figure reported by Folin and Ciocalteau.[8] Of the other proteins given in Table I, the percentage of tryptophane in edestin and egg albumin agrees with the values obtained by the latter workers. The data given in Table I show that such mixed proteins as those of muscle should be incubated for a longer period than the more simple proteins in order to obtain the maximum color intensity. This is probably due to the difficulty of effecting complete solution of the protein material which contains muscle fibre. In this connection Thomas[9] has reported that the tryptophane value of casein rose from 1.3 to 1.6% when the Herzfeld[5] method was used, and from 0.6 to 1.78% when the Fasal[10] method was employed. It is obvious, in order to obtain accurate results, that complete solution of the protein in question is a necessary prerequisite. In the case of finely ground proteins which are difficultly soluble, about 8 days of incubation are necessary in order to reach the maximum color intensity. It is advisable that daily comparisons of the sample solution be made after the sixth day in order to ascertain the point of maximum color intensity.

7719 C

Experimental Hypersensitivity to Undenatured H. Pertussis Protein.*

JOHN J. MILLER, JR.,† AND ALCOR BROWNE. (Introduced by A. P. Krueger.)

From the Department of Bacteriology, University of California, Berkeley.

It has been observed that hypersensitivity to a solution of undenatured *H. pertussis* protein[1] may occur during whooping cough.[2, 3] It was therefore considered of interest to determine whether anaphylaxis could be provoked with this agent in guinea pigs. Sensitization of these animals was attempted by injection of living *H. pertussis,* undenatured *H. pertussis* protein, and sera of animals immunized against *H. pertussis.*

8 Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627.

9 Thomas, P., *Ann. Inst. Past.*, 1920, **34**, 701.

10 Fasal, H., *Biochem. Z.*, 1912, **44**, 392.

* Partially supported by Grant-in-Aid of Research from Eli Lilly and Company.

† National Research Council Fellow in Medicine.

1 Kreuger, A. P., Nichols, V. C., and Frawley, J. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1097.

2 Stallings, M., and Nichols, V. C., *Am. J. Dis. Child.*, in press.

3 Frawley, J. M., Stallings, M., and Nichols, V. C., *J. Pediatrics*, 1934, **4**, 179.

The effect of undenatured *H. pertussis* protein on normal uterine strips and intracardially *in vivo* was first determined. Five females of 350-750 gm. weight were subjected to hysterectomy under ether anesthesia. (Removal of the uterus was done only between the 6th and 11th days of the oestral cycle.) The strips were washed in Locke's solution and then placed in a Dale bath of Locke's solution. When small rhythmic contractions began the test substance, *H. pertussis* protein in Locke's solution, was added to the bath. It was found that the addition of the test substance to make a final dilution of 1 part of protein in 100,000 regularly stimulated contraction of the strip. The contractions were immediate, at least two-thirds maximal and sustained for 4-8 minutes. They were indistinguishable from contractions produced by histamine. After washing, a second, third and fourth addition of *H. pertussis* protein in this dilution initiated similar contractions. Furthermore if larger doses were given no evidence suggestive of desensitization was obtained.

A day or two later the hysterectomized animals were given intracardial injections of undenatured *H. pertussis* protein in Locke's solution. It was found that doses up to 0.2 mg. could be given without systemic reactions. Larger doses were not given because of the highly dilute state of the protein solution available—1 part in 4,500.

Active sensitization with living H. pertussis. Four female guinea pigs of 300-450 gm. weight were injected with living Phase 1 *H. pertussis* bacilli intraperitoneally (3 day growth on Bordet-Gengou medium). Four injections were done at weekly intervals, the dose being increased from 1 billion to 10 billion organisms. Two days after the last injection one animal was found *in extremis*. Examination disclosed the typical hemorrhagic peritonitis.[4] The uterus failed to contract in the Dale bath on the addition of *H. pertussis* protein and gave only a slight reaction to histamine. Six to 7 weeks later the remaining animals were subjected to hysterectomy. Both uterine horns of one of these gave a positive Schultz-Dale reaction in a dilution of 1 part of *H. pertussis* protein in 7,200,000. The contractions were immediate, almost maximal and sustained 3 and 6 minutes respectively. After washing, subsequent additions of *H. pertussis* protein in this high dilution produced no contraction —indicating desensitization with the primary addition. When progressively larger amounts of the test substance were added no contractions occurred until the oxytocic dose (1 part protein in 100,000) was reached. Here repeated reactions occurred. It is felt that this

[4] Bordet, J., and Gengou, O., *Ann. de l'Inst. Pasteur*, 1909, **23**, 45.

animal's uterus was undoubtedly sensitized to *H. pertussis* protein. Confirmation by intracardial injection of protein was not obtained however. The animal died during a subsequent bronchospasm test.

The uteri of the 2 remaining animals of this group did not contract until a dilution of 1 part *H. pertussis* protein in 240,000 was reached. After washing, second additions of this dilution failed to produce contractions. Desensitization and hence sensitization is here suggested but as the dose was only slightly greater than the usual oxytocic dose (1 in 100,000), the results are equivocal. When these animals were injected intracardially with 0.2 mg. of *H. pertussis* protein anaphylactic death did not occur. Some dyspnea of questionable significance was noted. The intracardial dose of antigen here used is admittedly small but because of the high dilution of the preparation a larger dose could not be given intracardially.

Four other guinea pigs were given 5 injections of living *H. pertussis* intratracheally at 5-day intervals in doses increasing from 1 billion to 10 billion. The established inability of this organism to infect the guinea pig was here well illustrated. All of the animals gained weight during this period. The organism was recovered from the nasal secretions of only one. None developed pulmonary symptoms. Three to 4 weeks after the last injection hysterectomy was performed. Schultz-Dale tests were negative, no contractions occurring in dilution of protein higher than 1:100,000. No pulmonary lesions were found on post-mortem examination of the lungs.

Three other guinea pigs were given single subcutaneous injections of living *H. pertussis* and tested 3 to 8 weeks later. The uterus of one of them contracted and was desensitized at a dilution of 1 part protein in 240,000. The intracardial test was negative.

Sensitization with undenatured H. pertussis protein. To determine whether undenatured *H. pertussis* protein could induce anaphylactic hypersensitiveness 5 guinea pigs were given single subcutaneous injections of 0.06 to 0.2 mg. and tested 3 to 8 weeks later. The uterus of one of these animals tested 3 weeks after the injection gave the typical immediate and prolonged Schultz-Dale reaction to *H. pertussis* protein 1 part in 720,000. No subsequent addition of protein produced a contraction until the oxytocic dose of 1:100,000 was reached. Specific sensitization is therefore inferred. The intracardial injection of 0.2 mg. of *H. pertussis* protein did not, however, produce anaphylactic death. The uteri of the remaining animals of this group reacted only to the oxytocic dose.

Passive Sensitization. Passive sensitization could not be demon-

strated in 5 animals prepared by intraperitoneal injection of rabbit sera high in precipitin and complement fixing antibody titer. Tests were done one to 4 days after injection of the sera.

Summary. A solution of undenatured *H. pertussis* protein was found to be oxytocic in the Dale bath in high dilution. The normal guinea pig uterus reacted with a histamine-like contraction to repeated additions of *H. pertussis* protein—1 part in 100,000.

Active sensitization of the guinea pig uterus to undenatured *H. pertussis* protein was produced by repeated intraperitoneal injections of living Phase 1 *H. pertussis.* In one instance the classical Schultz-Dale reaction was obtained with 1 part of *H. pertussis* protein in 7,200,000. The uterine strips of 3 other animals prepared by injection of living *H. pertussis* contracted and were desensitized in dilution only slightly higher than oxytocic.

Active sensitization of the guinea pig uterus was also produced in one instance by a single subcutaneous injection of undenatured *H. pertussis* protein. The strip contracted and was desensitized by 1 part of *H. pertussis* protein in 720,000. Passive sensitization with *H. pertussis* immune sera of high titer was unsuccessful.

7720 P

Effect of Oestrin and Gonadotropic Hormone Injections upon Hypophysis of the Adult Rat.

WARREN O. NELSON. (Introduced by M. M. Ellis.)

From the Department of Anatomy, University of Missouri.

ERRATUM

In Article 7720, on Effect of Oestrin and Gonadotropic Hormone Injections upon Hypophysis, by W. O. Nelson, first line should read, "Moore and Price's studies have stimulated interest in the pituitary-gonadal relationships."

trated males, and [illegible]
with from 15 to 50 R. U. of oestrin, as Theelin in oil,* for 28 to 40 days; 22 males and 29 females injected daily with 50 to 150 R. U. of Antuitrin-S for 14 to 35 days; 11 males and 23 females injected daily with 15 R. U. of a sheep pituitary extract for 12 to 25 days;

[1] Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

* All of the hormone preparations used in this study were kindly supplied by Drs. O. Kamm and D. A. McGinty, Parke, Davis and Company.

and 5 males and 7 females injected daily with 30 to 40 R. U. of an extract of pregnant mare serum. In addition, the hypophyses from 13 castrated males, 11 spayed females, and 14 cryptorchid males which had received daily 50 to 100 R. U. of Antuitrin-S for 10 to 30 days have been studied.[2]

At the termination of an experiment the hypophyses were removed with the gonads and sex-accessories, weighed and fixed for study. For the hypophyses, Zenker-formol, Zenker-formol-acetic, Regaud's or Champy's fluids have been used. A modified Mallory, iron-hemotoxylin, Altmann's or Severinghaus' technique have been employed in staining these pituitaries.

There are certain differences in the hypophyseal picture, depending upon the amount and duration of treatment. The principal and characteristic findings are as follows: With the exception of castrates receiving Antuitrin-S, whose pituitaries were in no way altered,[2] the typical pictures in the hypophyses of all the above animals were very similar. The most striking effect has been a pronounced decrease in the number of normal basophiles. In the castrates (oestrin treated) and cryptorchids there has been an almost complete disappearance of the so-called "castration cells". In the Zenker and Regaud preparations there is a great increase in the number of cells which appear to be chromophobes.[3] However, in the Golgi preparations many of these apparent chromophobes are seen to be degranulated basophiles with enlarged Golgi rings and numerous mitochondria. The acidophiles are more erratic in their behavior, but in general they, too, show a decrease in normal cells with the appearance of degranulated forms. Similar findings have been recorded by Severinghaus,[4] using Follutein.†

A quantitative difference occurred in the male and female pituitaries. A considerably greater amount of hormone is required to obtain a comparable picture in the male gland. This is in keeping with the observation[5] that the male hypophysis is much less easily influenced by gonad hormone than the female gland. As a rule the female gland increases in weight, an observation less constant in the male.

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[2] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1192.

[3] Nelson, W. O., *Anat. Rec.*, 1933, **55**, 70.

[4] Severinghaus, A. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 593.

† Since this paper was originally submitted an additional paper by Severinghaus[6] has appeared.

[5] Nelson, W. O., *Anat. Rec.*, 1933, **55**, 31.

[6] Severinghaus, A. E., *Anat. Rec.*, 1934, **60**, 43.

The similarity in effects produced by oestrin and by gonadotropic preparations in both sexes suggests the possibility that the active factor in animals receiving gonadotropic material has been oestrin produced by the animals' own gonads. This is almost certainly true in the female and is not unlikely in the male.

Some of the degranulated basophiles seen in the Golgi preparations have much the same appearance as the so-called "pregnancy cells". These pregnancy cells have been considered generally to be chromophobes or acidophiles modified through the activity of the corpus luteum of pregnancy. From present findings it seems possible that the pregnancy cells are responsible for the maintenance of the corpus luteum of pregnancy rather than genetically through the action of the corpus luteum hormone.

Our findings are open to different interpretations. It may be considered that the increased gland weight and the large number of degranulated basophiles indicate an increased secretory activity on the part of the basophiles. The enlarged Golgi, numerous mitochondria, and prominent nucleoli all might be taken as evidence of such a functional state. However, we cannot always ascribe physiological activity purely on the basis of a morphological picture and in the present instance the physiological findings are not indicative of basophilic activity. Judged solely on the basis of the pituitary findings in the animals injected with gonadotropic hormone it is difficult to determine the functional condition of the pituitary since the sexual organs, whose condition are an index of the gonadotropic activity of the pituitary, are directly stimulated by the injected hormone. When the gonads and sex accessories (male) of animals injected with oestrin are considered, a different condition is observed. Although the hypophyses present the same morphological picture as in animals receiving gonadotropic hormone, the gonads, and through them, the accessories are very apparently damaged. This is believed to be due to the direct action of oestrin on the hypophysis.[1] Implantation studies and blood and urine examinations offer additional evidence that secretory activity of the hypophysis is inhibited by oestrin. The morphological picture in the hypophysis, which in some instances is distinctly abnormal, indicates that there has been a severe derangement of normal cell activity occasioned by an upset of the normal hormonal balance between gonads and hypophysis. A more exact statement concerning the nature of these altered relations and their resultant effect on pituitary activity must await further experimentation.

7721 P

Metabolism of Methionine in a Case of Cystinuria.

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The metabolism of dl-methionine in cystinuria has been studied in a male cystinuric (age 14 years) using a normal subject of the same age and sex as a control. The oxidation of l-cystine has also been compared with that of racemized cystine. While both are readily oxidized, the urinary results indicated slightly more efficient oxidation of the l-cystine, confirming the work of duVigneaud, *et al.*[1]

Administration of dl-methionine equivalent to 0.500 gm. S with urine collection for the following 48 hours results in an increase in sulfate sulfur corresponding to approximately two-thirds of the methionine administered with a simultaneous increase in unoxidized sulfur. The excretion of the extra sulfur by the cystinuric is much slower than by the control. In the cystinuric, no significant increase in the cystine output was observed by the Sullivan method.[2]

Methionine determinations[3] were run on all urines. Normal urines give a small titration by this method and both normal and the cystinuric subject showed 4 to 6 fold increases in the titration during the 24 hours following methionine ingestion. However, the absolute amount of the increase accounts for not more than 10 to 15% of the methionine sulfur. With both subjects, the increase in unoxidized sulfur was too great to be accounted for by the methionine increase. The urine of the normal subject after methionine administration gave negative cyanide-nitroprusside tests.

Prolonged daily administration of 10 gm. sodium bicarbonate (or its equivalent in sodium citrate) over a period of months has not changed the cystine output.

Administration of equimolar amounts of glycine and glutamic acid for a period of one week was also without effect on cystine excretion.

The increase noted by Brand, Harris and Biloon[4] in the appar-

[1] duVigneaud, V., Craft, H. A., and Loring, H. S., *J. Biol. Chem.*, 1934, **104**, 81.

[2] Brand, E., Cahill, G. F., and Harris, M. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 348.

[3] Baernstein, H. D., *J. Biol. Chem.*, 1934, **106**, 451.

[4] Brand, E., Harris, M. M., and Biloon, S., *J. Biol. Chem.*, 1930, **86**, 315.

ent cystine content of these urines on standing, as indicated by the Sullivan method, has also been observed by us.

7722 P

A Preliminary Analysis of the Spectra of Some Hemoglobin Derivatives.*

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The absorption spectra of oxyhemoglobin, carboxyhemoglobin, cyanhemoglobin, methemoglobin (pH 5.9) and methemoglobin (pH 9.2) have been studied quantitatively in both the visible and ultraviolet regions.

These pigments have complex absorption patterns. The absorption curves are all very different in the visible region, but exhibit a general similarity of shape in the ultraviolet where most of the light absorption is evident.

The absorption curves of certain far simpler substances have yielded to an analysis which resolves the complex of peaks and troughs into a series of curves (bands), whose summation gives the observed absorption pattern.[1] Bands whose peaks are at equal frequency distances from each other may be considered to possess an intimate relationship, and probably represent the same fundamental disturbance in the molecule caused by the absorption of energy. This is an important deduction since it greatly simplifies the interpretation of a complex absorption curve.

It is noteworthy that this type of analysis may be applied to the absorption curves of such complex molecules as these various hemoglobin derivatives. The spectrum of cyanhemoglobin is composed of a single series of bands, spaced at regular intervals. The absorption curves of the other pigments studied possess bands which belong to the same series, although they also show other bands. Cyanhemoglobin and oxyhemoglobin may be used as examples (Table I).

* This work was aided by a grant from the Faculty Research Fund of the University of Pennsylvania. Part of the work was carried out in the Eastman Laboratory of Physics of the Massachusetts Institute of Technology, by the kind permission of Professor George R. Harrison.

[1] Hagenbach, A., and Percy, R., *Helv. Chim. Acta*, 1922, **5**, 454. Brode, W. R., *Proc. Roy. Soc. (Lond.)*, A, 1928, **118**, 286.

TABLE I.

Uncorrected Position of Peaks				
Cyanhemoglobin		Oxyhemoglobin		
λ in mμ	$\nu \times 10^{-2}$	λ in mμ	$\nu \times 10^{-2}$	$\nu \times 10^{-2}$ in Multiples of 60
		575	174	
545	184	540	185	3
418	239	415	241	4
351	285	345	290	5
272	368	276	362	6
229*	437	240*	417	7

*Readings in this spectral region are uncertain.

This preliminary analysis permits the following tentative deductions to be drawn: All the hemoglobin derivatives have bands which belong to a single series, the members of which may be expressed by $n = \frac{\nu \times 10^{-2}}{60}$. n represents a simple integer, such as 3, 4, 5, 6, and 7, which are demonstrable in the regions of the spectra studied. This series of bands is probably related to the general structure of the hemoglobin molecule.

The so-called α-band of oxyhemoglobin (peak at λ 575 mμ) probably belongs to another series. Since a similar discrepant band is present also in the spectrum of carboxyhemoglobin this portion of the absorption curve may be related to the union of hemoglobin with O_2 or CO.

Upon the basis of this analysis the possible existence of absorption in the infra-red may be prophesied. In the case of cyanhemoglobin, for example, the first 2 members of the $\frac{\nu \times 10^{-2}}{60}$ series would be expected at approximately λ 1660 mμ and λ 830 mμ.

7723 C

Velocity of Blood Flow as Influenced by Exercise and Increased Air Pressure.

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In a series of studies to determine the effect of increased air pressure on the human body, encountered in deep sea diving, one of the most important things to determine is the velocity of blood flow during rest and exercise at normal and increased air pressures.

TABLE I.

Subject	Atmospheres Pressure Used	Condition	Pulse	Blood Pressure	Pulse Pressure	Circulation Time in sec.	Injection Time in sec.	Actual Circulation Time in sec.
1	1	Rest	62	116/60	56	16	3	13
		Exercise	104	136/68	68	16	4	12
	6	Rest	70	108/64	44	23	3	20
		Exercise	74	114/72	42	24	4	20
2	1	Rest	60	116/68	48	19	3	16
		Exercise	100	142/48	94	13	3	10
	6	Rest	70	128/72	56	19	3	16
		Exercise	78	144/86	58	20	3	17
3	1	Rest	80	96/50	46	18	3	15
		Exercise	110	124/50	74	12	3	9
	6	Rest	96	114/52	62	14	3	11
		Exercise	92	116/42	74	13	4	9
4	1	Rest	82	110/60	50	17	3	14
		Exercise	110	124/30	94	14	3	11
	6	Rest	72	102/80	22	13	3	10
		Exercise	88	128/74	54	15	3	12
5	1	Rest	78	126/76	50	18	4	14
		Exercise	128	130/70	60	14	3	11
	6	Rest	80	124/44	80	17	3	14
		Exercise	96	136/66	70	18	3	15
6	1	Rest	82	112/48	64	15	3	12
		Exercise	120	130/18	112	10	3	7
	6	Rest	92	112/70	42	11	2	9
		Exercise	80	130/58	72	14	3	11
7	1	Rest	76	114/64	50	16	2	14
		Exercise	108	182/42	140	15	5	10
	6	Rest	78	110/62	48	20	2	18
		Exercise	96	138/82	56	14	2	12
8	1	Rest	60	120/72	48	19	2	17
		Exercise	78	126/68	58	14	4	10
	6	Rest	54	132/56	76	19	4	15
		Exercise	72	112/56	56	19	3	16
9	1	Rest	58	102/52	50	15	3	12
		Exercise	112	164/30	134	11	3	8
	6	Rest	72	104/72	32	19	6	13
		Exercise	96	120/58	62	13	4	9
10	1	Rest	62	88/60	28	16	3	13
		Exercise	102	136/34	102	15	3	12
	6	Rest	72	92/68	24	15	6	9
		Exercise	84	112/56	56	18	3	15
11	1	Rest	54	110/72	38	20	5	15
		Exercise	96	122/74	48	15	4	11
	6	Rest	64	110/75	35	22	6	16
		Exercise	74	88/76	12	20	3	17
12	1	Rest	64	114/68	46	19	3	16
		Exercise	92	132/36	96	17	5	12
	6	Rest	59	112/58	54	21	2	19
		Exercise	86	120/70	50	15	3	12
13	1	Rest	62	124/62	62	20	2	18
		Exercise	88	136/88	48	19	3	16
	6	Rest	64	126/76	50	20	3	17
		Exercise	66	150/68	82	28	4	24
Aver.	1	Rest	68	111/63	48	17.5	3	14.5
		Exercise	104	137/51	86	14.2	3.5	10.7
	6	Rest	73	113/65	48	17.9	3.5	14.4
		Exercise	83	124/67	57	17.8	3.2	14.6

Various methods* for measuring the rate of blood flow have been described. The method of Winternitz and his associates[1] as modified by Gargill[2] was found most suitable. These investigators used an aqueous solution of sodium dehydrocholate, marketed as "Decholin", as the test material for intravenous injection.

Six cc. of the content of a 10 cc. ampule of a 20% solution of sodium dehydrocholate were aspirated into a sterile 10 cc. syringe using an 18 gauge needle. A vein in the antecubital space of the arm was entered and 3 cc. of the solution were injected as rapidly as possible. The time taken for injection as well as the time of onset of the bitter taste on the end of the tongue were registered by means of a stop watch. After an interval of one or 2 minutes, with the needle still in place, another 3 cc. of the solution was injected and the velocity of blood flow was again measured. Second injections were not made at 6 atmospheres pressure due to the rapidity of blood clotting when under increased air pressure.

Observations were made upon resting subjects and upon the same subjects after standard exercise, *i. e.*, subject standing, squatted on his heels simultaneously raising his arms to a horizontal position and returned to normal standing position 20 times in 30 seconds. Observations were then made upon the same subjects at 6 atmospheres air pressure under resting and exercise conditions as outlined above. All observations were made with the subjects in the recumbent position. The injections were made within 10 seconds after exercise.

Pulse and blood pressure were taken on the resting subjects just before the injections of "Decholin" were made. Pulse and blood pressure were taken immediately after standard exercise on all subjects. When the pulse and blood pressure had returned to normal the subjects again exercised and the injections were made.

Thirteen men accustomed to working under increased air pressure acted as subjects in these experiments. The complete results of this study are given in Table I. The velocity of blood flow is shown graphically in Chart 1.

The velocity of blood flow as measured by the arm to tongue circulation time was determined at atmospheric pressure with the subjects resting and was found to average 14.5 seconds, with 12 seconds the fastest and 18 seconds the slowest rate of flow measured. These results compare favorably with those reported by

* Bibliography, see Gargill.

[1] Winternitz, M., Deutsch, J., and Brull, Z., *Med. Klin.*, 1931, **27**, 986.

[2] Gargill, S. L., *New Eng. J. Med.*, 1933, **209**, 1089.

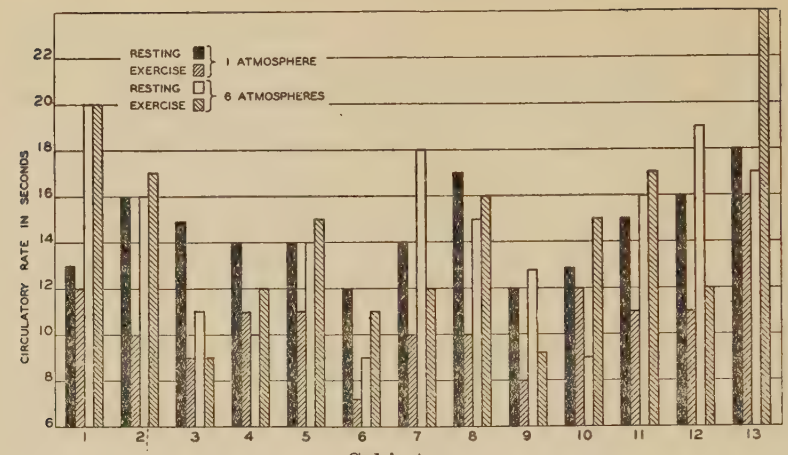

CHART 1.

other investigators using either the "Decholin" or the radioactive method. Gargill[2] using "Decholin" in 50 subjects reported an average actual circulation time of 15 seconds.

After exercise at atmospheric pressure the rate of flow was faster in all subjects. The average was 10.7 seconds, with 7 seconds the fastest and 16 seconds the slowest measured. The average rate of flow was 3.8 seconds faster than with the subjects in a resting condition.

The average pulse in resting subjects was 68 but after exercise the average pulse was 104. The average pulse pressure of resting subjects was 48 which increased to 86 after exercise. Thus as would be expected it was found that pulse, pulse pressure and velocity of blood flow increased after exercise at atmospheric pressure, and this increase was noted in all the subjects.

The velocity of blood flow at a pressure of 6 atmospheres was measured in the same subjects at rest and was found to average 14.4 seconds with 9 seconds the fastest and 24 seconds the slowest rate of flow measured. Although this was the same average as that found in resting subjects the variation among the subjects was greater. The increased air pressure did not affect the blood flow of all the subjects in the same way for the rate of flow was increased in 3 subjects, remained the same in 6 and decreased in 4 subjects. Neither did it affect the pulse nor pulse pressure in the same way in all individuals for in some it increased and in some it decreased.

The average rate of flow in the same subjects after exercise at 6 atmospheres pressure was 14.6 seconds, 7 seconds being the fastest

and 24 seconds the slowest rate of flow measured. This average was 3.9 seconds slower than after the same standard exercise at one atmosphere, and was practically the same as the average rate of blood flow in the subjects at rest either at one or 6 atmospheres pressure.

The effect of air pressure on the blood flow of the subjects after exercise was even more marked than on the resting subjects. Unlike exercise at one atmosphere the rate of blood flow did not increase in all the subjects. The rate of blood flow was increased by exercise in only 4 subjects, remained the same in one, was apparently slower in 4 and was decidedly slower in 4 other subjects. Neither did the pulse nor pulse pressure show the same degree of change after exercise at 6 atmospheres that it did after exercise at one atmosphere; the increase in the average pulse rate (Table I) was but 10 per minute following exercise at 6 atmospheres while following exercise at one atmosphere the increase in the average pulse rate was 36 per minute. The increase in the pulse pressure following exercise at 6 atmospheres was but 9 mm. Hg. while at atmospheric pressure the increase in the pulse pressure was 38 mm. Hg. Neither the pulse nor the pulse pressure was increased in all subjects, but decreased in some. It is thus shown that exercise at 6 atmospheres pressure does not have the same stimulating effect on the circulatory system that it has at one atmosphere.

Cardiac outputs as calculated by the formula of Fürst and Soetbeer[3] indicate that the minute volume of a resting subject at atmospheric pressure in most cases decreases when the same subject is at rest under increased air pressure. Although these calculations are empirical it is apparent that studies of cardiac output under increased air pressure are important. Further studies of minute volume under increased air pressure are now in progress.

Summary and conclusions. 1. Data have been presented showing the velocity of blood flow, pulse and blood pressure on 13 subjects while at rest and following exercise both at one and 6 atmospheres pressure. 2. Velocity of blood flow, pulse and pulse pressure increased following exercise at one atmosphere. 3. The velocity of blood flow was increased in 5, remained the same in 4 and decreased in 4 resting subjects at 6 atmospheres pressure. 4. Exercise at 6 atmospheres pressure does not have the same stimulating effect on the circulatory system that it has at one atmosphere. 5. Six atmospheres of pressure does not have the same effect on the velocity of blood flow in different individuals either at rest or after exercise.

[3] Fürst, T., and Soetbeer, F., *Deut. Arch. fur Klin. Med.*, 1907, **90**, 190.

7724 P

Use of Helium as a New Therapeutic Gas.

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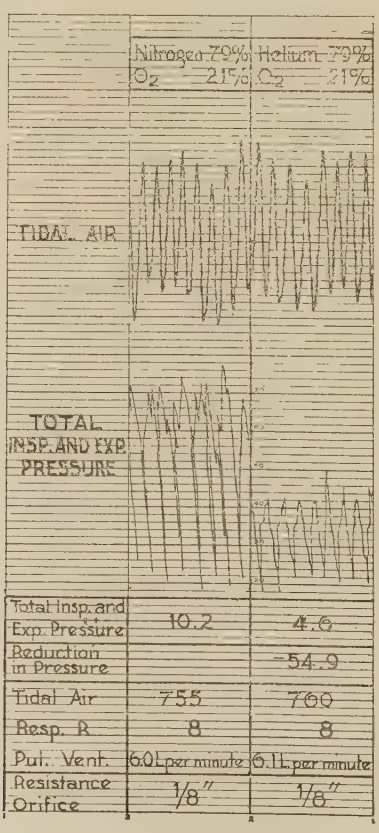
With an atomic weight of 4 and a density of 0.138, helium is the lightest of all the gases except hydrogen. The explosive possibilities of the latter render it too dangerous for clinical use. When helium is substituted for nitrogen in the air, the specific gravity of the mixture (21% oxygen and 79% helium) is 0.341, as compared to that of air. 1. The helium-oxygen mixture is 66% lighter. Since *work* is in general proportional to the density, the pressure required to move helium-oxygen mixtures in and out of the lung should be decidedly less than nitrogen-oxygen mixtures.



Graph 1.

An "artificial lung" was constructed by using a spirometer in which the bell was moved up by a motor running at constant speed and pressure, and lowered by constant weights. The spirometer "breather" (was filled by) different mixtures of gases from a Douglas bag or from another spirometer. The volume of air entering and leaving the spirometer (the tidal air) was graphically recorded on a kymographic drum. The pressure of the air within the spirometer, corresponding to a theoretical intra-pulmonary pressure, was obtained by making a leak-tight connection into the spirometer bell and connecting it to a water manometer, which then recorded the lifting or inspiratory pressure and the compressing or expiratory pressure. When the helium-oxygen mixture was "respired", the tidal air was increased from 23% to 50%, with a reduction in pressure of from 2% to 21%.

The effect on patients with compensated heart disease of breathing through a slightly narrowed orifice was measured. The inspiratory and expiratory pressures in the pulmonary air-ways were obtained by connecting a water manometer to the tube which came from the patient's mouth. They were then graphically recorded. Twenty observations on 10 such patients showed a consistent decrease in the pressure necessary to move a helium-oxygen mixture than to move air. The reduction of the total inspiratory and expiratory pressures was between 20% and 45%.

When a still smaller orifice was employed, there was a reduction in the total inspiratory and expiratory pressure of 54%. Observations were also made on patients breathing helium-oxygen mixtures without any narrowed orifice but simply against the resistance imposed by the spirometer bell and flutter valves. When mild dyspnea was produced in 4 compensated cardiac patients, a reduction in pressure in the pulmonary air-ways of 25 to 50% was obtained. In one cardiac patient who was slightly dyspneic at rest, the same phenomenon was recorded as in the patients who were made dyspneic by mild exercise, namely, a marked reduction (40%) in the pressure used in moving the helium-oxygen mixture in and out of the lung. In all the cardiac patients, there was observed corresponding to the decrease in pressure, a decrease in the total pulmonary ventilation when helium-oxygen mixtures were inhaled.

In a previous study,[1] helium as well as other rare gases were excluded from air atmospheres for periods as long as 6 weeks without apparent harm to animals (mice) living in these rare gas-free atmospheres. The biologic inertness of helium was further con-

[1] Barach, A. L., *Science*, December, 1934.

firmed by the fact that mice lived in an atmosphere of 21% oxygen and 79% helium without apparent injury for periods as long as 2½ months.

Sayers and Yant[2] exposed animals to 10 atmospheres of helium-oxygen mixtures and then decompressed them in ⅓ of the time necessary for animals exposed to similar nitrogen-oxygen mixtures. They suggested the use of helium and oxygen for caisson workers and divers exposed to excessively high barometric pressures because helium has a lower solubility and a greater diffusivity than nitrogen. The physical basis for the use of helium in clinical disease is its decreased specific gravity in comparison to nitrogen. According to the formula $F = Ma$, in which F is force, M mass and A acceleration, the movement of air requires a force three times that of a mixture of 20% oxygen and 80% helium. The velocity of gas mixtures diffusing through small orifices is proportional to the square root of the molecular weights, approximately twice as fast in the helium-oxygen mixtures than air. In conditions in which there is resistance to the movement of an adequate volume of air to and from the lungs in any part of the respiratory system from the pharynx to the alveoli, helium-oxygen mixtures may be transported with less effort. When large pulmonary ventilations are maintained over long periods of time, the saving of pulmonary effort is relatively great. Clinical studies in asthma, pneumonia and cardiac failure are in progress.

7725

Types of Specific Carbohydrates in the Cholera and Cholera-Like Vibrios.*

RICHARD W. LINTON AND B. N. MITRA.

From the All-India Institute of Hygiene and Public Health, Calcutta.

We reported the finding of 2 types of specific carbohydrate in the vibrios.[1] Both types contained an aldobionic acid from which on prolonged hydrolysis galactose and glucuronic acid could be

[2] Sayers, R. R., and Yant, W. P., *Anesthesia and Analgesia*, 1926, **5**, 127.

* This work was done with the support and under the auspices of the Indian Research Fund Association.

[1] Linton, Richard W., and Shrivastava, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 600; 1933, **31**, 406.

isolated. The first type, which has been found only in vibrios isolated from cholera, also contained a second sugar, which proved to be galactose. The second type of specific carbohydrate, which occurred in a few of the vibrios from cholera and in all of the non-agglutinating water vibrios, had arabinose in place of the galactose of the first type. Both galactose and arabinose were found to be readily separable from the specific substance by mild hydrolysis. The structure and constituents of these carbohydrates have been more extensively considered in other publications.[2]

As an example of the second type of carbohydrate and of the results of its analysis we may outline the study of vibrio W880, which was isolated from water in an endemic cholera area (Calcutta), and is a smooth, non-agglutinating organism. Three and one-half grams of the purified specific polysaccharide was added to 5.0 cc. of 50% H_2SO_4 and the mixture placed at 37° for 20 hours. Reducing substances, 18%, calculated as glucose. The sulphuric acid was removed from the hydrolysate by $CaCO_3$, and the calcium salt of the aldobionic acid prepared and precipitated out by the addition of alcohol. The supernatant fluid was concentrated and a phenylosazone prepared from it which had a melting point of 158° to 160°. On the basis of our previous experience with the vibrio carbohydrates, we were able to take this result as an indication of the presence of arabinose.[2]

The decomposed calcium salt was found to have a reducing power equivalent to 80 mg. of glucose. On hydrolysis on the steam bath it showed a gradual increase in reducing power up to 21 hours, when 252 mg. (219% increase) were present. At 23 hours a slight decline had occurred, and the heating was stopped. From the hydrolyzed material mucic acid (m.p. 222°) and potassium acid saccharate were prepared, indicating the presence of galactose, and under these conditions, of glucuronic acid in the complex.[2]

We have now found in certain vibrios a third type of specific carbohydrate. In studying a smooth, agglutinating vibrio from a case of cholera in Rangoon (designated Rangoon Smooth) we found that its specific carbohydrate was of the first kind described above, *i. e.*, it contained galactose, and an aldobionic acid of the usual type. From Rangoon Smooth a rough colony (Rangoon Rough (1) was isolated, which contained non-agglutinating organisms only. The carbohydrate of this strain proved to have the same structure as that of the smooth parent, but was present in only

[2] Linton, Richard W., and Shrivastava, D. L., *Indian J. Med. Res.*, 1933, **21**, 91, 379; January, 1935, in press.

about one-fifth the amount, *i. e.*, 1% as against 5% in the smooth form. That is, this rough strain had shown the usual phenomenon of roughening being accompanied by loss of specific carbohydrate which we have noted in the vibrios,[3] and which appears widespread among bacteria. From Rangoon Rough (1) a further organism was then isolated (designated as Rangoon Rough (2)) whose colonies were of the extremely rough "medusa-head" type, with a dry corrugated surface, extremely tenacious growth on agar, scanty growth in broth, slow fermentation of glucose alone out of the several sugars tried, loss of indol formation; and the strain was in every way a complete dissociant from the original Rangoon Smooth. Serologically also it appeared to have nothing in common with the parent strain. Although the mass growth on agar was extremely slow and poor, the percentage of specific carbohydrate which was present was the same as that found in the parent smooth strain, *i. e.*, 5%. The method for separating the polysaccharides from the vibrios has been reported previously.[2]

4.222 gm. of the gum from Rangoon Rough (2) were taken up in 10 cc. of 50% H_2SO_4 and kept at 37° for 18 hours. Reducing power, 10.9% (460 mg.) calculated as glucose. After dilution to bring the acid concentration to 3% the mixture was further hydrolyzed on the water bath for 2 hours; reducing power, 12.2% (514 mg.); a further heating for 2 hours gave 9.4% reducing power (415 mg.) and hydrolysis was stopped.

The hydrolysate was treated exactly as in the case of W880 carbohydrate. No precipitate of calcium aldobionate could be obtained. The solution presumably containing the aldobionic acid had a reducing value equivalent to 7.6 mg.; on hydrolysis this increased to 9.0 mg. after 2 hours and decreased to 6.0 mg. at 4 hours, when the experiment was terminated. In a second experiment, in which 4.0 gm. of the carbohydrate were used, the initial reducing power of the presumed aldobionic acid solution was *nil*, increased to 8.0 mg. after 2 hours heating and was again negative after 5 hours heating. It is probable that these small amounts represent reducing substance carried over into the presumed aldobionic acid solution from the hydrolysate. It was evident throughout these experiments that we were not dealing with a carbohydrate of the type we had previously found among the vibrios.

The portion of the hydrolysate remaining after the attempted separation of the calcium aldobionate was then studied. It yielded

[3] Linton, Richard W., Mitra, B. N., and Shrivastava, D. L., *Indian J. Med. Res.*, 1934, **21**, 749.

a phenylosazone melting sharply at 210°. It was concentrated *in vacuo* until it had a syrupy consistency, seeded with a few crystals of glucose, a little absolute alcohol added and the mixture placed in the refrigerator under vacuum. After 4 days a solid mass of white crystals had appeared. They were washed with a little ethyl alcohol, followed by methyl alcohol and finally with ethyl alcohol and dried *in vacuo*. Yield: 0.3350 gm.

0.15 gm. made up in distilled water to form a 1% solution, which was perfectly clear, gave a specific rotation of +54.0°.

The crystals did not have a definite melting point, but showed irregular changes between 110° and 125°. The phenylosazone melted sharply at 214°, and the melting point was unchanged when it was mixed with known glucose-phenylosazone. The characteristic crystals of potassium acid saccharate were also readily obtained.

From this evidence it appears possible to identify the substance as glucose, and to conclude that it is the sole constituent of the specific carbohydrate. The finding of a dissociant with a polysaccharide completely different from that of its parent strain is of some interest, especially in view of the fact that we have found by cross-absorption tests that the 2 vibrios are unrelated serologically, and as the succeeding paper will show are also different in their protein constituents.

The same type of glucose-containing polysaccharide has been found in another vibrio strain, Basrah II, a rough strain which was isolated along with 3 other strains from cholera cases in Basrah in 1931. It is a strain of considerable variability in its serological and biochemical reactions. Working with 4.2 gm. of specific polysaccharide of this organism by the methods outlined above, what appeared to be unequivocal evidence for the presence of glucose as the sole constituent of the carbohydrate was obtained, and as in Rangoon Rough (2) no aldobionic acid could be isolated.

It thus appears that at least 3 types of specific polysaccharide are present in the vibrios:

	Constituents	Source
Type I	Galactose + an aldobionic acid consisting of galactose and glucuronic acid.	In most vibrios from cholera cases.
Type II	Arabinose + an aldobionic acid consisting of galactose and glucuronic acid.	In a few vibrios from cholera cases, and in all the non-agglutinating water vibrios studied.
Type III	Glucose only; no aldobionic acid.	In a dissociant (Rangoon Rough (2), and in an aberrant strain (Basrah II).

What appears to have been the third type of specific carbohydrate was reported by Jermoljewa and Bujanowskaja[4] as having been isolated from an old Russian strain of *Vibrio cholerae*. The phenylosazone of this substance melted at 204° and the substance itself gave a specific rotation of +64.0° and was tentatively identified as glucose by these authors.

7726

Proteins and Carbohydrates of the Cholera and Cholera-Like Vibrios.*

RICHARD W. LINTON AND B. N. MITRA.

From the All-India Institute of Hygiene and Public Health, Calcutta.

The results of the van Slyke analyses of the proteins of the cholera and cholera-like vibrios, which we have published elsewhere,[1] may be briefly summarized. No differences in nitrogen distribution could be found between proteins of the agglutinating vibrios and of the non-agglutinating or water vibrios. In comparison with similar analyses of other bacteria, which have been reviewed by Hirsch,[2] the vibrios form a well-defined group with a relatively high content of the simpler amino acids (average, 55.7%) and a lower content of the basic amino-acids (average, 24.4%) than has previously been found in the bacteria. The figure for amide nitrogen, which averaged 6.8%, is about half that reported for other microorganisms. Taken altogether, the van Slyke analyses indicated that the vibrios had, relative to other bacteria, a comparatively simple structure, and as already stated the nitrogen distribution appeared identical in all of them from whatever source. An elementary analysis of the vibrio proteins was also made and in no case could any differences in these constituents be detected in the group.[3]

We have also studied the vibrio proteins by the method of "racemization" in dilute alkali, which was developed by Woodman[4] and

[4] Jermoljewa, Z. W., and Bujanowskaja, I. S., *Z. Immunitätsf.*, 1930, **68**, 346.

* This work was done with the support and under the auspices of the Indian Research Fund Association.

[1] Linton, Richard W., Mitra, B. N., and Shrivastava, D. L., *Indian J. Med. Res.*, 1934, **21**, 635.

[2] Hirsch, J., *Z. Hyg. and Infektionskr.*, 1931, **112**, 660.

[3] Linton, Richard W., Shrivastava, B. L., and Mitra, B. N., *Indian J. Med. Res.*, 1934, October, in press.

[4] Woodman, H. E., *Biochem. J.*, 1921, **15**, 187.

based on the earlier work of Dakin.[5] Woodman's method consists of following the optical activity of the protein in dilute alkali and plotting the degree of specific rotation against time, and was applied by him to the study of the globulins and albumins of cow sera and colostrum. Since it appeared from our previous work that the vibrios were closely related in their protein constituents, it seemed that this method might be applied to a possible differentiation of them. A typical example of the method and of the results obtained may be briefly outlined. Approximately 40 gm. of dried vibrio growth were collected, and the globulin separated out by repeated precipitations with ammonium sulphate. In no case was more than a trace of albumin present in any of the vibrios studied, and in most of them this constituent appeared to be absent. The globulin was dialyzed against running water followed by distilled water, until sulphates could no longer be detected, the pseudo- and euglobulin fractions separated, washed, dried and weighed.

The 2 globulins were then taken up in N/2 NaOH, to make a 1% solution, and polarimetric readings taken at intervals, using a 1 dm. tube and sodium light. No differences were found in the readings of the pseudo- and euglobulin solutions in any given vibrio. During the period of the readings the solutions were kept at 37°. The results obtained from the pseudoglobulins of a cholera vibrio (2027) and a water vibrio (W3075) in N/2 alkali are given in Table I.

TABLE I.
Specific Rotations of the Pseudoglobulins of Cholera Vibrio 2027 and Water Vibrio W3075 in N/2 NaOH.

Hours	Cholera Vibrio 2027 Specific Rotation	Water Vibrio W3075 Specific Rotation
1	—76°	—71°
5	—65°	—60°
24	—44°	—39°
48	—36°	—31°
96	—27°	—22°
120	—25°	—20°
145	—23°	—18°
196	—20°	—15°
217	—19°	—14°
264	—19°	—14°

After the period of about 200 hours the readings became constant and showed no further changes up to 350 hours, beyond which the experiment was not carried.

It is clear from the data given in Table I that the "racemization" of the 2 proteins begins, proceeds and ends differently, and it is

[5] Dakin, H. D., *J. Biol. Chem.*, 1912, **13**, 357.

probable that this difference may depend upon the differing structure of the 2, since the rate at which the change in rotation occurs will be an expression of the rate of change within the protein molecule and will vary with the structure of the molecule (Woodman,[4] Jordan-Lloyd[6]). In both cases the data yield a perfectly smooth curve. As we have already found that the nitrogen distribution is the same in both these proteins it would follow from these results that the difference observed might be due to the different manner in which the amino-acids were grouped together within the respective molecules of the two proteins.[7]

Altogether 20 vibrios have been studied by the "racemization" method, and we have found that these 2 curves are the only ones obtained. It should be emphasized that in the case of either kind of curve the agreement between similar proteins is extremely close, and the readings do not vary more than a degree and are usually

TABLE II.
Origin, Agglutinability, Protein, and Specific Carbohydrate Content of a Series of Cholera and Cholera-like Vibrios.

Number	Origin	Agglutinability	Protein No.	Carbohydrate No.*
1617	Cholera	Agglutinable	I	I
1676	"	"	I	I
79A	"	"	I	I
Rangoon Smooth	"	"	I	I
Rangoon Rough (1)	From Rangoon Smooth	Non-agglutinable	I	I
Rangoon Rough (2)	From Rangoon Rough (1)	"	II	III
2027	Cholera	Agglutinable	I	II
505	"	"	I	II
E	"	"	I	II
W880	Water	Non-agglutinable	II	II
W3075	"	"	II	II
79B	Cholera	Irregular	II	I
El Tor	Human; non-cholera	Agglutinable	II	I
" " I†	" "	"	II	I
" " II	" "	"	II	I
" " III	" "	"	II	I
Basrah I	Cholera	Irregular	II	I
" II	"	"	II	III
" III	"	"	II	I & III
" IV	"	"	II	II & III

*Carbohydrate No. I is the galactose-containing specific substance; No. II is the arabinose-containing; and No. III the glucose-containing. The details are given in the preceding paper.

†El Tor strains I, II, and III represent respectively strains Nos. 3657, 3658, and 3659 of the National Type Collection, London.

[6] Jordan-Lloyd, D., Chemistry of the Proteins, Churchill, London, 1926.

[7] Linton, Richard W., Mitra, B. N., and Shrivastava, D. L., *Indian J. Med. Res.*, 1934, **21**, 749.

identical. We have designated the protein giving the first type of data above (2027) as Protein I and the second protein (W3075) as Protein II. In Table II are given the results of the racemization method in relation to origin, agglutinability and type of specific carbohydrate in the series of vibrios studied.

In the preceding paper we have given an account of the Rangoon strains and their specific carbohydrates, and it is only necessary here to point out that the dissociant Rangoon Rough (2) belongs to the second protein group in contrast to its parent strains Rangoon Smooth and Rangoon Rough (1), and this finding taken in conjunction with the different types of specific carbohydrate present in the two is to be correlated with the complete non-identity of the 2 strains in cross-absorption tests. Rangoon Rough (2) had its origin from a colony picked from a plate streaked with Rangoon Rough (1). Whether it arose as a mutation or whether it was simply by the chance of streaking that a colony of this distinctive "medusa-head" type was isolated is of course impossible to determine. Rangoon Rough (2) is extremely slow in its growth, and would be easily overgrown and hidden under ordinary conditions when mixed with rapidly growing strains like Rangoon Smooth and Rangoon Rough (1). On the other hand, during the 12 months in which these 2 strains have been under continual observation in this laboratory, a colony like that of Rangoon Rough (2) has been observed only on the one occasion, a fact which might be considered to favor the mutation hypothesis.

Whichever idea of the origin may be the correct one, it is clear that from a smooth, agglutinating cholera vibrio there has been derived a rough non-agglutinating vibrio differing from the parent in having the protein constituent found in the water vibrios and an entirely distinct type of specific carbohydrate substance. At the same time the derived form is wholly different serologically from its parent form.

A further point of interest in the Table lies in the position occupied by the El Tor strains. As is well known, these strains have always been anomalous among the vibrios because they are agglutinating organisms isolated from human non-cholera cases under conditions of pilgrimage, where cholera outbreaks were to be expected, but have not in fact occurred. On the basis of their chemical structure the 4 El Tor strains in our series occupy an intermediate position between the cholera and the water vibrios; that is, their protein belongs to the same type as that of the water vibrios while their specific substance (Type I) is identical with that of the major-

ity of vibrios found in clinical cholera. Two other strains in the Table, both of them also variants from the type of vibrio generally found in cholera, show the same structure: 79B, which on isolation from a case of cholera in Calcutta was non-agglutinating, and which has since shown considerable irregularities in this reaction, and Basrah I which is also an irregularly agglutinating strain from cholera. It may be that the basis for the peculiarities of this group of 6 vibrios lies in the hybrid relationships of the protein and carbohydrate types of which they are composed.

The Basrah strains all possess the same kind of protein (Type II). The structure of Basrah I and II has been discussed above and in the preceding paper. Basrah III and Basrah IV are of especial interest because they appear to be mixed strains in the sense that 2 types of specific carbohydrate can be isolated from them. In the case of Basrah III the galactose- and glucose-containing specific substances are present, and in Basrah IV the arabinose- and glucose-containing types. These are the first mixed strains which we have found in an analysis of the carbohydrates of about 40 vibrios. It is probable that we are dealing here with 2 groups of organisms in the same culture, some of which have one and some the other of the 2 carbohydrates. From the repeated analyses of these vibrios which we have carried out it appears that the proportions of the organisms containing the 2 types of carbohydrate may vary from time to time.

As a result of the work on the proteins and carbohydrates of the vibrios it has become possible as we have shown to distinguish 2 types of protein and 3 types of specific carbohydrate in the group. On the basis of the various combinations which we have found of these constituents the vibrios in our series may be divided into several groups as follows:

Group I (Protein I and Specific Carbohydrate I) contains most of the vibrios isolated from clinical cholera.

Group II (Protein I and Specific Carbohydrate II) contains some of the vibrios found in clinical cholera. These are, however, somewhat less frequent than those of Group I. In its composition Group II is intermediate between the cholera vibrios of Group I and the non-agglutinating vibrios of Group III.

Group III (Protein II and Specific Carbohydrate II) contains the non-agglutinating water vibrios.

Group IV (Protein II and Specific Carbohydrate I) contains the El Tor strains and at least 2 other aberrant vibrios.

Group V (Protein II and Specific Carbohydrate III) contains at

present only 2 vibrios, the dissociant Rangoon Rough (2) and Basrah II.

Group VI (Protein I and Specific Carbohydrate III). Of this combination no examples have as yet been found, but their existence among dissociants of the other groups is not improbable.

7727 C

Pantothenic Acid Content of Animal Tissues.

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Evidence has been presented[1] for the existence in all types of living tissue of a substance which has been named "pantothenic acid". A quantitative biological test based upon yeast growth which is specific for this substance has been developed.

While marked progress has been made in this laboratory in concentrating and purifying the acid it is unlikely that any chemical method for its determination can be devised for some time to come. In order to learn something of its functions, however, it seemed desirable to obtain approximate information as to the content of various animal tissues.

Each of the tissues indicated below was thoroughly ground and extracted with a large volume of hot water, usually 100 times its weight. The pantothenic acid which is not "bound" in the tissues is thus extracted, and that which in some cases, at least, is "bound" is not determined. The numerical values are based upon the pantothenic acid extracted from a unit weight of moist tissue, in comparison with that in a unit weight of an arbitrary standard preparation. This "standard" was prepared by extracting rice bran with 60% methanol and evaporating to dryness. Our most potent concentrate is approximately 8,000 times as effective on a weight basis as this standard.

Duplicate or triplicate determinations were made in every case. These usually agreed within about 10%. In order to save space, only averages are given: Skeletal muscle dog No. 1, 0.032, dog No. 2, 0.037, rat, fresh, 0.034, rat autolyzed at 37°, 0.147; smooth

[1] Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.*, 1933, **55**, 2912.

muscle, dog, 0.033; heart muscle, dog No. 1, 0.032, dog No. 2, 0.020, sheep 0.053; skin, dog, 0.007; blood vessel, dog, 0.0012; duodenum, dog, 0.020 stomach, antrum, dog, 0.008 fundus, dog, 0.008; lymph gland, dog, 0.011; blood, dog, 0.0021; human, 0.005; spleen, dog, 0.01, ovary, dog, 0.004, testis, dog 0.014, cockerel, 0.09; liver, dog No. 1, not taken at once, 0.153 dog No. 2 (placed in methanol immediately), 0.008, sheep (few hours after butchering), 0.09, same allowed to autolyze at 37°, 0.30, rat taken immediately, 0.036, same in ice box 3 days, 0.20, same autolyzed at 37°, 0.45; human (autopsy), 0.07, cancerous portion, 0.0045, cockerel, not taken at once, 0.19; adrenal, dog, 0.046, beef, 0.058, cortex, beef, 0.063; kidney, dog, 0.036, lung, dog, 0.007; pituitary, whole, dog, 0.027, post. beef, 0.026, ant. beef, 0.025; brain, whole, dog No. 1, 0.081, rat, taken immediately, 0.036, same in ice box 3 days 0.062, sheep, few hours after butchering, 0.054, grey matter, dog No. 2, 0.08, white matter, 0.067; umbilical cord, human, 0.005; thyroid, dog No. 1, 0.013, dog No. 2, 0.008; pancreas dog No. 1, 0.040, dog No. 2, 0.027; fatty tissue, dog, 0.0014.

The following conclusions may be drawn: The acid is present in determinable amounts in all animal tissues. Where tissues from several animals have been examined there are roughly parallel values for these tissues. The richest tissue is liver. If taken very fresh, however, the liver has a relatively low value which may increase by more than 12 times by autolysis. This indicated that the acid is stored in the liver in a non-extractable form. Some storage is likewise indicated in muscles.

It appears that pantothenic acid is not peculiarly concerned in the fabrication of any specialized hormone but is a common constituent of all types of cells. Experimental work to be published elsewhere is in line with the conclusion that this substance is a *sine qua non* of respiration in general and that the universal presence is explained on this basis.

7728 P

Treatment of Adult Scurvy with Crystalline Vitamin C (Ascorbic Acid).

IRVING SHERWOOD WRIGHT. (Introduced by W. J. Stainsby.)

From the Second Medical Division, Bellevue Hospital and Department of Medicine, Cornell University Medical School, New York.

Three patients suffering from severe adult scurvy, with the classical physical findings, have been treated with the use of Crystalline Vitamin C (ascorbic acid). They were kept on a Vitamin C poor diet during treatment. A rapid improvement was noted within 6 days in each instance and a complete restoration to normalcy obtained within 4 to 5 weeks. The criteria utilized in determining improvement were:

(a) Improvement in general condition.
(b) Disappearance of purpuric spots and cessation of the appearance of new purpuric spots.
(c) Cessation of bleeding from nose, gums and intestines.
(d) Marked decrease in capillary fragility to within normal limits.

The capillary fragility test used was a standardized modification of the tourniquet test, performed as follows: A circle with a diameter of 2.5 cm. was drawn on the inner aspect of the forearm, 4 cm. below the elbow fold. The cuff of a Baumanometer was applied above the elbow, and the pressure maintained between the diastolic and the systolic pressure for 15 minutes, then released; the number of petechial hemorrhages produced within the circle was noted with the naked eye.

The dosage used in the treatment of these patients was: 0.06 to 0.09 gm. per day orally. Other patients have been treated with ascorbic acid administered intravenously, up to 0.1 gm. per day, dissolved in 5 cc. of sterile normal saline. No untoward effects have been noted from the intravenous use of this vitamin.

7729 P

Relationship between Gastric Juice Volume and Erythropoiesis in Patients with Untreated Pernicious Anemia.

S. MILTON GOLDHAMER. (Introduced by Raphael Isaacs.)

From the Thomas Henry Simpson Memorial Institute, University of Michigan, Ann Arbor.

Qualitative studies of gastric secretion of patients with pernicious anemia suggest that the so-called "intrinsic factor" of Castle is present, but in amounts less than normal.[1] These observations can explain why patients with pernicious anemia are able to mature some red blood cells, but does not answer the question of why various patients with pernicious anemia in relapse have different red blood cell levels. The present data show the relationship between the total amount of gastric secretion and the number of red

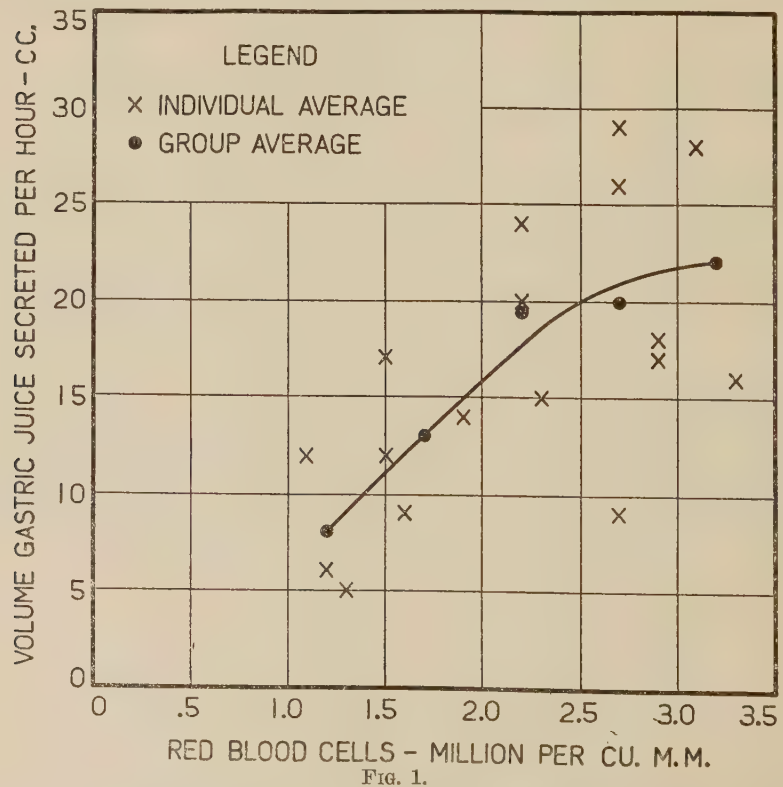

FIG. 1.

[1] Isaacs, R., and Goldhamer, S. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 706.

blood cells which can mature beyond the megaloblast stage and assume the adult characteristics in untreated patients.

The volume of gastric juice* secreted per hour and the red blood cell counts were determined in 17 patients with pernicious anemia in relapse. A total of 117 observations were made. Whereas the average gastric secretion for normal individuals is approximately 150 cc. per hour, the gastric secretion in patients with pernicious anemia was found to vary between 5 and 29 cc. per hour. If the average red blood cell count (ordinate) Fig. 1, is compared to the volume of gastric secretion per hour (abscissa), a direct relationship becomes apparent. That is, the greater the gastric secretion, the higher the red blood cell level. Apparently erythropoiesis depends in part, at least, upon the action of the intrinsic factor and the rate of red blood cell formation is related to the amount of the intrinsic factor produced.

Furthermore, there was suggestive evidence that as the gastric secretion was depleted by constant drainage, in each patient there was a decrease in the red blood cell count of the peripheral blood. These experiments are being repeated and amplified with various modifications to verify the conclusions.

7730 P

Creatine Mobilization in Myocardial Damage.

GEORGE HERRMANN AND GEORGE DECHERD.

With some technical assistance from Philippa Klippel.

From the University of Texas, Department of Medicine, Galveston.

In our clinical studies[1] the discovery of a hypercreatinemia and creatinuria following coronary thrombosis with cardiac infarction and in acute myocardial insufficiency with congestive failure prompted us to attempt to establish a curve of creatine excretion.

Old male dogs that showed no creatinuria, on a diet of 150 gm. of dried bread and 200 cc. of evaporated milk with chopped cabbage

* Gastric juice was obtained by continuous suction for 3-hour periods. Histamine hydrochloride injections did not influence the volume of secretion, and, therefore, was not used routinely. All specimens were obtained at least 6 hours after the ingestion of food.

[1] Herrmann, G., Decherd, G., Erhard, P., and Klippel, P., *J. Lab. and Clin. Med.*, in press.

and charcoal, were selected and varying grades of obstruction to the coronary circulation were produced with a minimal amount of skeletal muscle trauma. Under amytal anesthesia the right carotid artery was carefully exposed by a separation of the muscles, a lead impregnated ureteral catheter was guided, under the fluoroscope, through an opening in the carotid down into one of the aortic sinuses. With the tip opposite a coronary orifice a large or a small drop of metallic mercury was run in from a syringe. Thirty grams of glucose in 200 cc. evaporated milk was given by tube immediately after operation and repeated in 12 hours, after which the dog usually took all of his diet. A constant intake of food was maintained. A flash roentgenogram established the position of the mercury in the coronary system. Electrocardiograms taken before and at intervals following the injection presented graphic evidence of myocardial infarction.

In a series of 12 dogs a creatinuria developed within a day except in the very slight infarctions in which it sometimes appeared on the second or third day. The creatinuria then increased as is shown in Table I of Dog No. 12, to reach its maximum about the sixth

TABLE I.
Dog No. 12. Daily Excretion.

Days post-op.	Creatinine, mg.	Total mg.	Creatine, mg.	Blood Creatine, mg. %
Control	343	343	0	
Control	300	300	0	.58
1	342	342	0	.74
2	364	364	0	.94
3	384	397	13	.37
4	377	407	30	.49
5	329	373	44	
6	324	378	54	.55
7	331	363	32	
8	410	427	17	
9	365	372	7	.44
10	232	235	3	
11	315	315	0	.49
12	245	245	0	
13	257	257	0	

day. It then dropped off to disappear according to the extent of the damage between the tenth and fourteenth day. The nitroprusside test for acetone was constantly negative. In the instances of massive infarction hypercreatinemia appeared within 12 hours, and lasted 24-48 hours, and the creatinuria was more marked. The curves vary in contour and in height in proportion to the degree of muscle damage and are of some diagnostic and prognostic significance.

7731 P

An Experimental Study of the So-Called Liver Death Syndrome in Biliary Surgery.

FREDERICK FITZHERBERT BOYCE. (Introduced by Howard H. Beard.)

From the Department of Surgery, Louisiana State University Medical Center.

A series of experiments was undertaken with the idea of reproducing in experimental animals the clinical and postmortem findings observed in so-called "liver deaths" after biliary surgery, to which Heyd[1] first called attention. Such deaths fall into 2 distinct groups. In the first group death occurs shortly after operation, hyperpyrexia is the outstanding symptom, and degenerative liver changes are the only notable autopsy finding. In the second group death is deferred for 10 to 14 days, uremic symptoms predominate, and autopsy reveals the liver changes just described, plus similar degenerative changes in the convoluted tubules of the kidney. None of the usual surgical causes of death is apparent.

Ten sets of experiments were performed upon dogs whose renal competency was assured by detailed preoperative studies. The following experiments failed to reproduce the clinical and postmortem pictures desired:

1. Traumatic necrosis of the liver by various manual and instrumental methods. Eight dogs.
2. Various types of interference with the hepatic and portal circulation.* Sixteen dogs.
3. Obstruction of the biliary tree by ligation and division of the common duct, with or without cholecystectomy. Twelve dogs.
4. Intraperitoneal implantation of normal liver, as done by Andrews and Hrdina[2] and previous workers. Approximately 150 gm. of liver was implanted in each of 5 dogs.
5. Intraperitoneal injection of normal liver, as described by the same authors, who were able by this method to cause an autolytic peritonitis as in experiment 4, a finding which we could not confirm. Three dogs were used. Three hundred grams ground liver was extracted in 3,000 cc. of warm sterile water and concentrated to 17, 18.5 and 25 cc. respectively.
6. Intravenous injection of 20 cc. of extract of normal liver, extracted and concentrated in the proportion and by the method just described. One dog.

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[1] Heyd, C. G., *Ann. Surg.*, 1924, **79**, 55.

* A detailed study of this group of experiments will shortly be published.

[2] Andrews, E., and Hrdina, L., *Surg. Gynec. Obst.*, 1931, **52**, 61.

The following experiments were wholly or partially successful:

7. Obstruction of the biliary tree, as described in experiment 3 was continued 12 to 20 days and then released in 8 of the dogs which survived the first operation. The clinical picture of the second group of liver-kidney deaths was reproduced in all cases. Urinalysis and blood chemistry showed the changes characteristic of an acute renal condition, the blood nonprotein-nitrogen rising in 5 cases to 72, 81, 93, 105, and 171 mg. per 100 cc. All the animals died in uremic coma or were sacrificed when their condition was terminal and all the autopsies revealed degenerative changes in the liver and kidney corresponding to the histologic picture seen in human patients.

8. Intravenous and intraperitoneal extracts of the livers of the dogs in experiment 7 were injected in 10 other dogs, but did not reproduce the desired changes. One hundred thirty grams of liver was extracted in 130 cc. of sterile water, normal saline solution and 95% alcohol respectively, the alcoholic extract being concentrated to 13 cc.

9. Intravenous and intraperitoneal injection of saline and water extracts made by the above method in 6 dogs in which an attempt had been made to produce liver necrosis by chemical methods (the use of a carbon tetrachloride mixture). This experiment was likewise unsuccessful, but in the light of our other studies it now seems highly probable that experiment 8 failed because the extracts were not sufficiently concentrated, and that experiment 9 failed for the same reason, and because so long a time elapsed between production of the liver damage and injection that regeneration could have occurred.

10. Intraperitoneal injection with the water and saline extract of the liver of a patient who died a typical hyperpyrexia death after cholecystectomy produced the clinical and histologic picture characteristic of the liver-kidney syndrome in human patients, the degenerative changes in the liver and in the convoluted tubules of the kidneys being very marked. Similar injections with alcoholic extract of the same liver did not reproduce these changes. Three dogs. All the extracts were made in the same proportion, 130 gm. of liver to 150 cc. of sterile water, normal saline solution, and 95% alcohol respectively, and 30, 50, and 75 cc. of the extract were injected on successive days in each dog.

From these experiments and in the light of clinical studies elsewhere reported,[3] it is concluded: 1. The release of biliary obstruc-

[3] Boyce, F. F., and McFetridge, E. M., *Arch. Surg.*, in press.

tion rather than the obstruction itself is responsible for the fatal outcome in some cases of biliary surgery. 2. The hepatic changes always present in biliary disease are aggravated in some cases by the surgery instituted to relieve it, which imposes upon an already damaged liver an intolerable burden. As a result of the degenerative hepatic changes, there is released into the circulation a toxic substance which is presumed to be a water-soluble foreign protein. 3. When, after liver function fails, the kidney takes up the function of detoxification, there is resulting damage to its convoluted tubules, through which foreign proteins are excreted. 4. The liver-kidney syndrome is a single pathologic process, of which the kidney pathology represents the second stage.

Further experiments are at present being undertaken to support the hypothesis herein set forth.

7732 P

Precipitation and Complement-Fixation Reactions with Pneumococcus Soluble Specific Substance.

RACHEL BROWN.

From the Division of Laboratories and Research, New York State Department of Health, Albany.

The following observations, while but part of a broader investigation of the complement-fixation test with specific bacterial antigens in the diagnosis of infection,[1–4] have, nevertheless, a special significance in that they indicate the relative sensitivity of precipitation and of complement fixation with a highly purified bacterial antigen. The reports of other observers have been based upon studies with more complex antigens.[5–7]

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[1] Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *J. Immunol.*, 1925, **10**, 241.

[2] Wadsworth, Augustus. In: The Newer Knowledge of Bacteriology and Immunology, Jordan and Falk, editors, Chicago, University of Chicago Press, 1928, p. 831.

[3] Wadsworth, A. B., van Amstel, J. E., and Brigham, M. W., *J. Immunol.*, 1930, **19**, 289.

[4] Rice, C. E., *J. Immunol.*, 1932, **22**, 67.

[5] Dean, H. R., *Z. f. Immunitäts.*, 1912, **13**, 84.

[6] Parker, J. T., *J. Immunol.*, 1923, **8**, 223.

[7] Goldsworthy, N. E., *J. Path. and Bact.*, 1928, **31**, 220.

In both the precipitation and complement-fixation experiments, the soluble specific substance of the pneumococcus, Type I, was used as antigen with the pooled sera of 3 rabbits which had been immunized with formalinized Type I pneumococcus vaccine. The antigen was diluted 1:1000, 1:10,000, 1:100,000, 1:1,000,000, and 1:10,000,000. The serum was used undiluted and diluted with physiological salt solution 1:5, 1:10, 1:20, 1:40, and 1:80, with all of the antigen dilutions. For the precipitation tests, 0.3 cc. of antigen was mixed with 0.3 cc. of antiserum, and the reactions recorded according to the bulk of the precipitate after 2 hours' incubation at 37°C., followed by 24 hours' at ice-box temperature. The complement titrations were performed and the data calculated according to the method of Wadsworth, Maltaner, and Maltaner.[8] The tests were repeated 3 times, the complement-fixation with 3 different pools of complement.

For convenience, the precipitation and complement-fixation data are presented in one figure, although the units are not comparable.

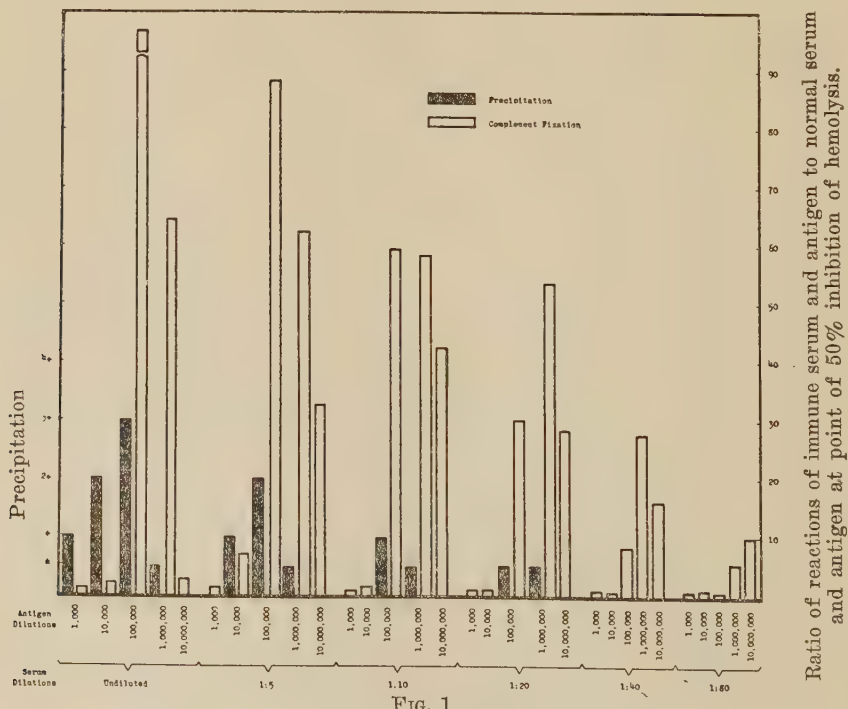

FIG. 1.

Relative sensitivity of precipitation and of complement fixation with pneumococcus soluble specific substance.

[8] Wadsworth, Augustus, Maltaner, Elizabeth, and Maltaner, Frank, *J. Immunol.*, 1931, **21**, 313.

The precipitation reaction was recorded as 4+, 3+, 2+, +, ±, and —. As the basis of comparison for the complement-fixation reactions, the ratio of the amount of complement necessary to give 50% inhibition of hemolysis in the presence of immune serum and antigen to the amount of complement giving the same reaction in the presence of normal serum and antigen, was used. In one instance (antigen dilution 1:100,000 and undiluted antiserum), such a ratio could not be determined as fixation was almost complete with the largest amount of complement used, namely 0.1 cc.

When the precipitation and complement-fixation data are compared, it is apparent that under the conditions of the tests the maximum reactivity occurred with the same antigen and antibody dilutions in both tests, namely, 1:100,000 with undiluted antiserum. The sensitivity of the complement-fixation reaction, however, exceeded that of the precipitation. With any one dilution of serum a broader range of antigen dilutions reacted strongly in titrations of complement than in precipitation tests. The direct proportionality between the amounts of serum and complement fixed in the specific reaction with tubercle-bacillus antigen and antiserum recorded by Wadsworth, Maltaner, and Maltaner was also observed in this reaction between pneumococcus soluble specific substance and anti-pneumococcus serum within the zone of optimum reactivity.

Summary. The results of the 2 tests, precipitation and complement-fixation, paralleled one another so closely with this highly purified soluble specific substance of Type I pneumococcus that the reactions appear to represent union of the same antigen and antibody. The comparative titration of complement, however, is the more sensitive measure of the specific activity of an immune serum. Within the zone of optimum reactivity the amount of complement fixed was directly proportional to the amount of immune serum present.

Peiping Section.

Peiping Union Medical College, October 24, 1934.

7733 P

Amount of Thyroid-stimulating Hormone in Anterior Pituitary of the Thyroidectomized Rabbit.

GRAHAM CH'EN AND H. B. VAN DYKE.

From the Department of Pharmacology, Peiping Union Medical College.

Although gonadectomy in the rat is followed by an increase in the amount of gonad-stimulating principle in the anterior pituitary (Engle, Evans and Simpson) it is said that thyroidectomy does not cause any change in the amount of thyroid-stimulating hormone in the anterior pituitary of the rat, guinea pig and dog.[1, 2] In our experiments we have compared the concentration and, in a preliminary way, the total amount of thyroid-stimulating hormone in the anterior lobes of thyroidectomized and control rabbits.

Thirty-one male rabbits (14 completely thyroidectomized and 17 control animals) and 28 female rabbits (10 completely thyroidectomized and 18 control animals) were used. There were available litter-mate controls for 7 of the thyroidectomized males and for 6 of the thyroidectomized females; other controls were from the same stock and of approximately the same age as the operated rabbits. The average time elapsing between thyroidectomy and death was about 4 months. The amount of thyroid-stimulating hormone in the anterior pituitary of a thyroidectomized rabbit was compared with that in the anterior pituitary of a control by injecting the same dose (usually 6 to 8 mg.) over a period of 4 days into litter-mate immature guinea pigs which were killed 24 hours after the last injection (on the 5th day) for histological examination of the thyroids. In some experiments, the doses injected were proportional to the weights of the donors' anterior lobes. In control experiments

[1] Houssay, Novelli and Sammartino, *C. R. Soc. Biol.*, 1932, **3**, 830.

[2] Hohlweg and Junkmann, *Pflüger's Arch.*, 1933, **232**, 148.

total doses of 4 mg. of fresh rabbit anterior lobe could be shown to stimulate the thyroid of the immature guinea pig.

In only 36.8 ± 9.11% of the pairs of guinea pigs receiving male anterior lobe, a greater thyroid-stimulation was caused by the anterior lobes of thyroidectomized rabbits. On the other hand, in 71.5 ± 9.85% of the paired guinea pigs receiving female anterior lobe, the thyroid-stimulation was greater in the guinea pigs receiving anterior lobe from thyroidectomized rabbits. The difference appears to be significant (34.7 ± 13.42%). However, thyroidectomy in the rabbit commonly causes a hypertrophy greater in the anterior lobe of the male than in that of the female (in our series: male, 166% of control; female, 133% of control); therefore, the *relative* dose, in terms of the control rabbit's pituitary, was greater in the case of the female group when the same absolute doses of anterior pituitary from control and thyroidectomized rabbits were used. In 5 groups of litter-mate male rabbits and 3 groups of litter-mate females, the doses of anterior pituitary were based on anterior pituitary weights. In 45 ± 15.0% of the paired guinea pigs, the male thyroidectomized rabbits' anterior lobes caused the greater stimulation, whereas in 72 ± 17.0%, the female thyroidectomized rabbits' anterior lobes caused the greater stimulation. The difference between these groups (27 ± 22.7%) is not significant. Whether or not thyroidectomy brings about an increase in the total amount of thyroid-stimulating principle greater in the anterior lobe of the female than in that of the male can only be decided by additional experiments which are under way.

7734 C

Further Studies on the Effect of Supersonic Waves on Bacteria.

SZU-CHIH LIU AND ALBERT C. H. YEN. (Introduced by Hsien Wu.)

From the Departments of Biochemistry and Bacteriology and Immunology, Peiping Union Medical College, Peiping.

We have shown[1] that exposure to supersonic waves brings about killing and dissolution of certain bacteria. The question as to whether these effects are due to the mechanical waves motions in the medium or cavitation of the dissolved gases remained unan-

[1] Yen, A. C. H., and Liu, S. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1250.

swered. The observations of the present study on the effect of supersonic waves on *B. dysenteriae* Shiga and *B. coli* suspended in gas-free solutions and in solutions saturated with air or hydrogen seem to indicate that the killing and dissolution of the bacteria is due to the cavitation of the dissolved gases.

The apparatus used for the generation of the supersonic waves at the rate of 1.5×10^6 times per second was the same as described by Wu and Liu.[2] One cc. of the bacterial suspension to be exposed was placed in a pyrex tube (20 mm. in diameter) containing a glass cooling coil through which cold water circulated. The temperature of the bacterial suspension throughout the entire experiment was always below 20°C., thus eliminating the possibility of destruction of bacteria by heat. Twenty-four-hour cultures of *B. dysenteriae* Shiga and *B. coli* on agar slant were washed and suspended in saline. To make the suspension gas-free, it was placed in the test tube and subjected to suction with a vacuum pump until the mercury manometer showed a constant minimum reading of 18 to 20 mm. The rubber tubing connecting between the test tube and pump was clamped and disconnected from the pump. The test tube containing the gas-free suspension under vacuum was then exposed to supersonic waves. To obtain hydrogen saturated suspension, hydrogen gas was allowed to pass through a sterile glass tubing with a cotton plug into the gas-free suspension under vacuum until one atmospheric pressure was reached. The tube was then shaken for 2 minutes to assure complete saturation with the gas, and the rubber tubing at the mouth of the test tube was clamped. The hydrogen saturated suspension thus prepared was then exposed to supersonic waves. The number of viable bacteria per cc. and the relative opacity of the suspensions before and after a 90-minute exposure to the supersonic waves were determined. The number of viable bacteria per cc. was determined by counting colonies in poured plates, while the relative opacity was determined with a Pulfrich photometer and expressed in per cent of the standard opacity glass NO_4. The results are summarized in Table I.

It will be noted from the table that the exposure of the bacterial suspensions to supersonic waves was followed by a reduction in number of viable bacteria and a decrease in opacity only when the suspension was saturated with air or hydrogen gas. These effects were more marked when the suspension was saturated with air than with hydrogen gas. We are not certain whether this difference is merely due to the larger quantity of the dissolved gases or the oc-

[2] Wu, H., and Liu, S. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 782.

TABLE I.

Number of viable bacteria per cc. and relative opacity of *B. dysenteriae* Shiga and *B. coli* suspensions before and after exposure to supersonic waves under different gaseous environments.

		Air saturated		Gas-free		Hydrogen saturated	
		Colony per cc.	Opacity %	Colony per cc.	Opacity %	Colony per cc.	Opacity %
B. dysenteriae Shiga	Initial	50.0×10^4	28.9	48.0×10^4	28.9	46.0×10^4	28.5
	90 min. control	48.0×10^4	28.9	41.0×10^4	28.1	42.0×10^4	28.2
	90 min. exposed	0	15.6	*55.0×10^4	28.2	96.0×10^2	21.4
B. coli	Initial	47.8×10^7	31.3	51.0×10^7	31.5	48.7×10^5	31.4
	90 min. control	46.3×10^7	31.3	48.0×10^7	31.5	44.8×10^7	31.2
	90 min. exposed	16.9×10^4	14.4	*58.0×10^7	31.3	24.0×10^5	18.8

*These 2 numbers are definitely higher than their corresponding initial or control colony counts. We interpret that this apparent increase is due to a more thorough shaking rather than actual multiplication of the bacteria in the suspensions.

currence of oxidation in case of air saturated suspension, but it is clear that cavitation of dissolved gases are essential in bringing about killing and dissolution of bacteria.

7735 C

Photometric Study of Bacteriophage Action.

F. C. LIN. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

Krueger and Northrop[1] were the first to undertake the study of bacteriophage action from a quantitative point of view. Based on extremely elaborate technical procedures, involving frequent and separate enumeration of bacteria and of bacteriophage throughout each experiment, they concluded that production of phage was proportional to a power of bacterial growth and that lysis set in almost explosively at the moment when phage/bacteria ratio attained a definite critical value. It would appear that according to their result the time required for lysis of a given bacterial concentration is proportional to the dilution of phage. Other conditions being constant, the strength of any 2 phages can be compared by noting the relative length of time necessary for reduction of a constant concentration of bacteria to an arbitrary end point.

The present paper is not intended to add anything new to the mechanism of bacteriophage action, but rather to present an accurate though considerably simpler device for attacking the problem from the same viewpoint. The apparatus used is a Pulfrich photometer which works on the principle of the Tyndall phenomenon, so that for a given bacterium, the number of organisms per cc. can be read off directly and quickly and is expressed in terms of percentage of a given standard. Very minute particles, namely, phage or protein particles, liberated during the process of dissolution of bacteria present a very weak Tyndall phenomenon and do not, therefore, affect the readings. Provided the bacterial suspension is not so thick as to interfere with penetration of light, the accuracy of readings obtained approached $\pm 0.5\%$ of the given standard, disregarding slight variations in the size and thickness of the tubes used.

[1] Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1931, **14**, 223, 493.

The phage used throughout the experiment is from d'Herelle's laboratory and has been in our stock since 1927. Sixteen to 18 hours' agar culture of a single strain of *B. dysenteriae* Shiga was suspended in meat-infusion broth, pH 7.6, and distributed in 9 cc. lots to a series of test tubes, to each of which 1 cc. of various dilutions of phage in saline was later added. With a view of securing uniform distribution of bacteria and phage, the suspensions were incubated in a mechanical shaker in a water bath at 32°C. Readings were taken every few minutes till the completion of lysis.

The results of a large number of experiments conducted with similar or modified techniques such as checking bacterial growth by limiting the source of nutritional supply, by substituting saline for broth, or by employing a thick initial bacterial suspension, or omitting constant shaking, all bore out the same conclusions which may be conveniently represented by the 3 accompanying graphs for (1) various dilutions of phage against a constant bacterial concentration, (2) constant dilution of phage against various bacterial concentra-

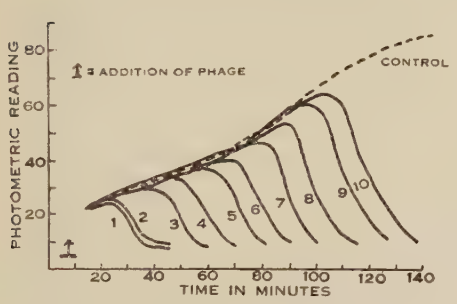

FIG. 1.

Various dilutions of phage (10^{-1}-10^{-10}) against constant bacterial concentration.

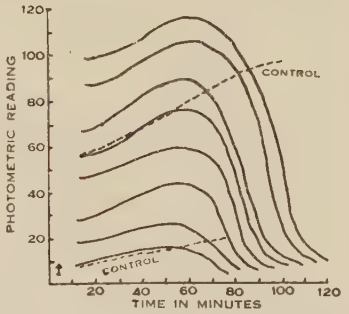

FIG. 2.

Constant dilution of phage (10^{-4}) against various concentrations of bacteria.

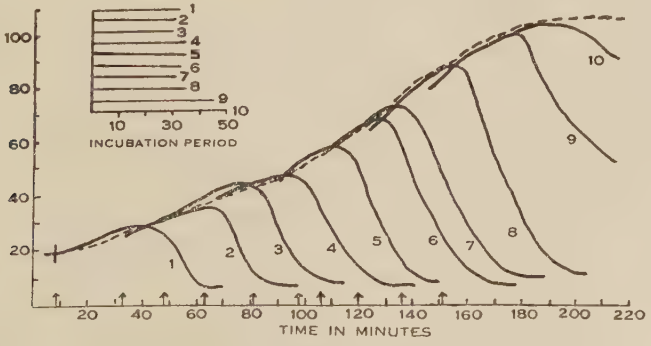

FIG. 3.

Constant dilution of phage (10^{-4}) added to constant initial bacterial concentration at various intervals of growth period.

tions and (3) constant dilution of phage added to constant initial bacterial concentration at different intervals of growth period.

Fig. 1 brings out the fact that with constant bacterial concentration, the incubation period (period between addition of phage and onset of lysis) is a function of dilution of phage, so that by plotting concentrations of phage as abscissa and incubation period in minutes as ordinate, a straight line connecting all points is obtained, except for very high concentration of phage. The latter is due to the fact that no matter how concentrated the phage is, there is always bound to be a minimal incubation period. When the experiment was repeated, a different slope was met with, but a similar curve conforming to a straight line is always obtained. It is interesting to note that within wide limits, the difference in the initial bacterial concentration does not influence the incubation period. (Fig. 2). This is to be expected, considering that production of phage is dependent on the growth of bacteria and that since a thicker suspension contains more bacteria in the process of active division, the increase of bacteriophage should also be proportionately greater. On the other hand as may be seen from the curve, the greater the concentration of bacteria at the end of incubation period, the longer the time required for clearing up of the culture. Here again the relation is that of a direct proportionality.

That the rate of production of phage depends on the multiplication of bacteria is also brought out in Fig. 3. Here, one may note that as growth of bacteria begins to slow down, the incubation period is also lengthened. Moreover, lysis of bacteria ushered in at that period is usually prolonged and frequently incomplete. It is well known that bacteria which have passed the stage of active growth become more resistant to the action of phage or perhaps a certain number of bacteria have died off; in the latter case they are not only refractory to the lytic action of phage but are able to remove the greater number of phage particles from the surrounding broth than would living, susceptible bacteria.

The above observations indicate that it is possible to apply the Pulfrich photometer to the study of bacteriophage action with the same precision as that obtained through the use of more intricate methods hitherto described by others.

7736 C

Preparation of the Specific Soluble Substance from Vaccinia Virus.

W. K. CH'EN. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

The presence of a specific precipitable substance in tissues infected with vaccinia virus has long been known. The nature of this substance remains, however, obscure. Recently Craigie[1] and Smith[2] reported on the preparation of a comparatively pure fluid extract from vaccinia virus. The extract exhibited definite immunological specificity, on the basis of which Smith regarded the substance responsible for this specificity as analogous in its behavior to the bacterial haptenes. If such be the case, it would seem reasonable to attempt its isolation in a form which would permit more or less accurate determination of the main characteristics of the substance.

The present communication deals with the successful experiment along this line. The technic used here was essentially that described by Smith with certain modifications. The precipitable substance was prepared both from vaccinia virus culture in River's medium consisting of a suspension of chick embryo tissue in Tyrode's solution and from testicular vaccinia virus (lapine). Control substances were also prepared from chick embryo tissue medium and from the testes of normal rabbits. To obtain a necessary supply of vaccinia virus culture, large Erlenmeyer flasks were used. Each flask contained from 25 to 50 cc. of culture. To avoid possible bacterial contamination of the medium during the process of inoculation, a horizontal short and narrow glass neck was attached to each flask near its bottom. Inoculation of flasks was done through this narrow glass neck.

In all instances the procedure of the preparation of the substance was the same. Infected tissue was ground in a glass mortar operated by motor until a very fine and homogeneous emulsion was obtained. In case of the testicular vaccinia virus or normal rabbit testes 5% suspension of the tissue in normal saline was used.

To the suspension 5% ether was added, to prevent deterioration of the tissue due to bacterial growth. The whole was then placed in an incubator for 7 days to allow autolysis to take place. Following this the suspension was centrifuged and the supernatant fluid

[1] Craigie, J., *Brit. J. Exp. Path.*, 1932, **13**, 259.

[2] Smith, W., *Brit. J. Exp. Path.*, 1932, **13**, 434.

passed through Seitz filter. The clear filtrate was adjusted to pH 5.5 and boiled for 5 minutes. This resulted in the formation of a heavy precipitate which was removed by centrifugalization and filtration. The solution was then adjusted to pH 8.0 and boiled again. Usually slight turbidity developed and this again necessitated filtration through Seitz filter.

After this the solution was brought up to the point of neutrality and evaporated over a water bath to one-third of its original volume. If any cloudiness appeared it was filtered off. To such a solution 9 volumes of mixture containing equal parts of ether and absolute alcohol were added, and the mixture was left in an ice-box over night. The white precipitate formed in the solution was collected, dried over a water bath, redissolved in distilled water and dialyzed in a cellophane bag for 48 hours in running water. After dialysis the solution was clarified by filtration and the dissolved substance thrown down by addition of 9 volumes of absolute alcohol. The precipitate was again collected and the whole procedure of precipitation with alcohol repeated. After 3 such precipitations a white and easily soluble substance was obtained. The yield was in average from 5 to 10 mg. per 10 gm. of the testicular vaccinia virus or per 200 cc. of the vaccinia virus culture.

Similarly a white and soluble substance was prepared from the control lots of the normal testicular tissue of a rabbit and chick embryo tissue medium. In the latter 2 cases the yield was, however, considerably smaller than that obtained in cases of infected tissues.

Chemical tests performed with each of 4 substances gave the following result: Biuret test negative, Ninhydrin test slightly positive and Molisch test strongly positive. Each substance was hydrolyzed in the presence of 2-N sulphuric acid and showed positive Benedict's test for reducing sugars. Both the substance prepared from the vaccinia virus culture and that derived from the testicular vaccinia virus were tested for antigenic power through the intravenous injections into rabbits. Subsequent examination of the sera of these animals showed no detectable antibodies. Both substances were also tested for their power to produce positive skin reaction in rabbits previously vaccinated or hyperimmunized with vaccinia virus. In all instances this test was negative. Although both experimental and control substances appeared to be similar so far as the above mentioned chemical tests were concerned, their behavior in the serological test was entirely different.

For this test rabbit anti-sera were prepared by vaccination, using

single intradermal injection of vaccinia virus culture, or testicular vaccinia virus or calf lymph. One month after such injection the sera were collected and used in the experiment as convalescent sera. The same rabbits were then immunized with vaccinia virus culture or testicular vaccinia virus using 4 intravenous injections each consisting of 1 cc. of the material given at weekly intervals. The sera obtained from such immunized animals were used here as hyperimmune sera. Control sera prepared through the immunization of rabbits with a suspension of normal testicular tissue of a rabbit or chick embryo tissue medium and serum from a normal rabbit were used in the experiment to rule out possible non-specific reactions.

The serological test consisted of precipitin and complement fixation reactions. Precipitin ring test was performed using various dilutions of each of 4 substances in normal saline against undiluted sera. In complement fixation test Kolmer's technic was followed with the only modification that a constant amount of the serum diluted 1 :10 was tested against different concentrations of the substances.

TABLE I.

Results of Precipitin and Complement Fixation Reactions Observed with S.S.S. Prepared from Vaccinia Virus.

		Soluble specific substances			
Anti-sera	Method of preparing anti-sera	Vaccinia virus culture	Testicular vaccinia virus	Chick embryo tissue medium	Normal rabbit testis
Hyperimmune	Injections of vaccinia virus culture	1:640 *1:800*	1:320 *1:800*	1:80 *Not tested*	0 *0*
"	Injections of testicular vaccinia virus	1:320 *1:800*	1:640 *1:1600*	0 *0*	0 *0*
"	Injections of chick embryo tissue medium	1:80 *Not tested*	0 *0*	1:160 *1:200*	0 *0*
"	Injections of normal rabbit testis	0 *0*	0 *0*	0 *0*	0 *0*
Convalescent	Single intradermal injection of vaccinia virus culture	1:160 *1:400*	1:160 *1:400*	0 *0*	0 *0*
"	Single intradermal injection of testicular vaccinia virus	1:160 *1:400*	1:160 *1:400*	0 *0*	0 *0*
"	Single intradermal injection of calf-lymph	1:160 *1:400*	1:160 *1:400*	0 *0*	0 *0*
Normal rabbit serum		0 *0*	0 *0*	0 *0*	0 *0*

Upper figures for precipitin titre; lower figures (in italics) for complement fixation titre; 0 denotes negative result.

The result secured in these 2 serological tests is given in Table I. As it is seen from the table positive precipitin and complement fixation reactions occurred regularly when specific antibody and antigen were brought into contact. In our case the possibility of non-specific reactions is excluded by various controls which were always negative. It was found that somewhat higher specific titre was recorded both in precipitin and complement fixation reactions when the antigen used in the tests was derived from a homologous material. It is only natural that higher titres for both reactions were observed when hyperimmune sera were used. It is to be noted that the reactions occurring in case of the antigen prepared from vaccinia virus culture and homologous anti-serum exhibited considerably higher titre than the same reactions developing in case of the substance derived from chick embryo tissue culture and the same serum. In this particular case one would expect to obtain non-specific result. This fact indicates that the precipitin and complement fixation reactions as reported in this work were mainly due to the activity of the specific substance derived from virus bodies. The specificity of the reaction is furthermore indicated by positive results obtained in case of substances prepared both from vaccinia virus culture and testicular vaccinia virus and anti-serum prepared through vaccination of a rabbit with calf lymph.

In the present work evidence was produced supporting the view that the precipitin reaction observed by several workers with extracts from the tissue infected with vaccinia virus and homologous anti-serum is caused by the presence in such extracts of a specific product of the vaccinia virus which can be isolated in the form of a polysaccharide similar in its behavior to the bacterial carbohydrate haptenes.

7737 P

Intermediate Hosts of Microfilaria Malayi in Chekiang, China.

LAN-CHOU FENG. (Introduced by R. J. C. Hoeppli.)

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Of the 2 species of human filaria found in China *Filaria bancrofti* is transmitted by *Anopheles hyrcanus* var. *sinensis, Culex pipiens* and *Culex fatigans* according to the researches of Feng,[1] Hu[2] and

[1] Feng, L. C., *Am. J. Hyg.*, 1931, **14**, 502.

[2] Hu, S. M. K., *Chinese Med. J.*, 1933, **47**, 1359, 1367.

Manson.[3] *Microfilaria malayi* has only recently been found in China (Feng[4]). It is therefore interesting to find out which species of mosquito is the intermediate host of this parasite. In the summer of 1933 (July to August), experiments have been carried out in Huchow, Chekiang Province, with the local species of mosquitoes and *Microfilaria malayi.*

TABLE I.

Species of mosquitoes	No. mosquitoes fed	No. mosquitoes dissected 1st to 4th day		No. mosquitoes dissected 5th to 8th day			Remarks
		No. +	No. —	No. +	No. —	No. with mature microfilariae	
A. hyrcanus var. *sinensis*	88	44	0	44	0	30	Larvae developed well, large numbers in each mosquito. Mature larvae were found beginning 6th day. Of 30 mosquitoes with mature larvae, 14 in labium.
M. (Manson-ioides) uniformis	14	2	0	12	0	2	Larvae developed normally till 4th day, afterwards some remained small, became granular and degenerated. Small numbers reached maturity on 8th day. In one mosquito mature larvae in labium.
Armigeres obturbans	27	9	0	3	15	0	2 of the 3 positive mosquitoes dissected on 6th day. 1 and 2 nearly mature larvae found in thorax. In one mosquito larvae were enclosed in chitinous capsules.
Stegomyia albopictus	28	3	11	0	14	0	In the positive mosquitoes, filarial larvae were found both in stomach and thorax, they appeared healthy.
Culex pipiens	74	8	23	0	43	0	Larvae dead, not yet exsheathed, in stomach of 6 mosquitoes. One small sausage-shaped larva in thorax of each of other 2 mosquitoes.

NOTE: All the mosquitoes were kept at room temperature of from 29° to 32°C.

[3] Manson, P., *China Customs Med. Rep.*, 1878, **2**, 1.

[4] Feng, L. C., *Chinese Med. J.*, 1933, **47**, 168.

Ten species of mosquitoes were found in Huchow, but only 5 species have been used for the experimental purposes because the remaining, namely, *Culex pallidothorax, C. vichnui, C. mimeticus, C. (Lutzia) vorax,* and *C. (Lutzia) fuscana* were either uncommon or did not suck human blood. All mosquitoes, except *M. (Mansonioides) uniformis,* were bred from larvae or pupae in the laboratory. The results of these experiments are given in Table I.

From the table it will be seen that *Anopheles hyrcanus* var. *sinensis* is an excellent intermediate host for *Microfilaria malayi.* Under the room temperature of 29-32°C. (July to August) the microfilariae developed in this species of mosquito quite normally and they reached maturity from the 6th day after the infective meal. Labial infection has been found very frequently beginning from the 6th day. Out of 30 mosquitoes in which mature microfilariae were found, 14 harbored microfilariae in the labium.

Microfilariae reached maturity also in *M. (Mansonioides) uniformis.* But this species of mosquito is probably not as good an intermediate host as *A. hyrcanus* var. *sinensis* since only a small number of the ingested microfilariae completed development. Majority of the microfilariae stopped development from the 5th day and became granular and degenerated.

The other 3 species of mosquitoes, namely, *Culex pipiens, Aedes (Stegomyia) albopictus* and *Armigeres obturbans* are not intermediate hosts of *Microfilaria malayi* since the microfilaria died and disappeared either in the stomach or after they reached the thorax of the mosquito.

7738 P

On the Nature of the Specific Reacting Substance of B. proteus X19 in the Weil-Felix Reaction.

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Through methods commonly employed for the recovery of specific soluble substances (polysaccharides) it is possible to obtain from the X 19 strains of the proteus bacillus specific reactive substances which flocculate with antiproteus and typhus serum, (Lim and Kurotchkin,[1] White,[2] and Castaneda[3]). Castaneda[3] has also shown

[1] Lim, C. E., and Kurotchkin, T. J., *Nat. Med. J. China,* 1929, **15**, 6.
[2] White, P. B., *Brit. J. Exp. Path.,* 1933, **14**, 145.
[3] Castaneda, M. Ruiz, *J. Exp. Med.,* 1934, **60**, 119.

that suspensions of Mexican Rickettsia similarly treated will yield a specific reacting substance which gives the same precipitation reactions with typhus and antiproteus sera as do the polysaccharides extracted from *B. proteus X19*. It appears, then, that there is in *B. proteus X19* and typhus *Rickettsia* a common antigenic complex which is responsible for the Weil-Felix reaction. This paper corroborates the above reports and emphasizes White's findings in regard to the dual antigenic nature of the polysaccharidal substance obtainable from the proteus bacillus.

Thirty-four samples of human typhus sera have been tested against extracts of *B. proteus X19* (0-504 strain). All of these were purposely selected from late or convalescent cases. All of them agglutinated the test organism at titers above 1 to 640 at the time the flocculation tests were carried out.

Fourteen of these sera were tested against *B. proteus* extracts made by White's method (hot NaOH extraction with alcohol precipitation) and also against extracts as prepared by Castaneda according to the method of Heidelberger and Avery[4] which includes extraction in cold antiformin, glacial acetic acid precipitation, precipitation of the supernatant fluid with cold alcohol followed by treatment with ammonium sulfate.

Twenty other samples of serum were tested both with White's extract and extracts made according to Castaneda's second method, that of alcohol precipitation of concentrates of 8-day broth cultures of *B. proteus X19*.

Antigen dilutions from 1 to 10 up to 1 to 10,240 were mixed with equal volumes of the typhus serum diluted 1 to 2. The dilutions were incubated 2 hours at 55°C., followed by 18 hours in an ice box at 13°C.

In all tests flocculation was observed at antigen dilutions ranging from 1 to 2560 as high, in 2 cases, as 1 to 20,560. This demonstrates that extraction methods employed in obtaining polysaccharidal substances from other organisms will remove from *B. proteus X19* a specific soluble substance which flocculates with human typhus sera. Control tests using serum from typhoid, pneumonia and one tularemia case did not result in visible flocculation under the same conditions.

In testing the 3 extracts against antiproteus serum (agglutinating titer 1 to 10,240) we found, as did White, that antiproteus serum did not flocculate with the extract prepared by treating the proteus

[4] Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **40**, 301.

bacilli with hot alkali. Flocculation did occur with the extracts prepared according to Castaneda's methods.

In a smaller series of tests, White[2] reported essentially the same results. He, therefore, feels that the somatic complex of *B. proteus X19* presents 2 distinct serological factors: one labile in hot alkali and responsible for 0 agglutination in its own antiserum without having any part in the Weil-Felix reaction; and another stable in hot alkali and responsible for the Weil-Felix reaction.

As to the chemical nature of the reacting substance, Castaneda and White report that this substance gives a powerful Molisch reaction, withstands boiling in alcohol or water and a negative biuret reaction. The extracts we prepared from the 0-504 strain of *B. proteus X19* reacted in the same way.

In using the alkali extract, we observed that it is necessary to adjust the H ion concentration within the limits of pH 7.2 and pH 7.4. Extracts which are too acid or too alkaline are liable to spontaneous flocculation at the incubation temperatures we employed.

We had difficulty in controlling the potency of hot alkali extracts which, although subjected to the same treatment, did not yield as much reactive substance as did similar preparations. We are unable to offer any explanation for this. One-day cultures do not yield as much reactive substance as 3 to 4-day cultures at 37°C.

Our work does not permit us to draw conclusions as to time in the disease when the reacting antibody may appear in the patient's serum. For this study we purposely selected sera which had a high reactive quality as indicated by their agglutinating titer. From the few tests we made with serum taken shortly after the onset of the disease, it would appear that significant flocculating titer parallels that of agglutination.

7739 C

Morphological and Tinctorial Behavior of B. Leprae During its Adaptation to an In Vitro Habitat.

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Much has been written concerning the variations in morphology and amphoteric staining property with respect to acid-fastness for the supposedly cultivated Hansen bacillus of leprosy during that

period in which the organism is acquiring the power to grow saprophytically. Confusion and doubt over the authenticity of the *in vitro* culture have arisen, because of the curious results obtained by various workers. These results presuppose that the specific microbe of human leprosy is of such protean pleomorphism that it may alternately appear as a non-acid-fast diphtheroid, as both acid-fast and non-acid-fast strepothrix, as a chromogenic and non-chromogenic acid-fast bacillus.

Kedrowski,[1] Deycke,[2] Bayon,[3] *et al.,* believe that *B. leprae* is capable of extreme pleomorphism and change in tinctorial properties which explains the non-acid-fast streptothrical and filamentous "stages" reported by them for the initial cultivation. Recently, Denney[4] reports what he regards as biological evidence that the "globi" in the human leprous lesion may be analogous to the sporangial or zoogleic stage of certain higher bacteria or protozoa. Salle[5] claims that *B. leprae* in culture upon living tissue (Carrel's medium) multiplies very slowly and appears both as acid-fast and non-acid-fast diphtheroidal forms. However, when the culture is subplanted to ordinary nutrient media a luxuriant "orange" colored growth occurs, the individual bacilli of which are now non-acid-fast.

Thus the literature reports a bewildering number of "stages" and transformations for *B. leprae,* which do not conform with our conception of metamorphosis for a single microorganism. However, the Hansen bacillus of leprosy, like many other parasitic microorganismal species, may exhibit minor changes in morphology while adapting itself to a saprophytic existence.

To determine the true morphological and tinctorial behavior of *B. leprae* during its adaptation to live and multiply in an artificial medium, the subcutaneous leprous nodule from 4 cases of human leprosy was secured for study through the kindness of Dr. Denney, physician in charge of the National Leprosarium at Carville, Louisiana. Each nodule was rich in the acid-fast-rods of Hansen and free from any cultivatable contaminant.

The leprous material was prepared and handled in the following way: (1) parts of each nodule were fixed in Zenker's fluid, paraffin embedded, sectioned and stained for acid-fast organisms; (2) emulsified bits of leproma were first treated with 1% solution of

[1] Kedrowski, *Z. f. Hyg. u. Infektionskr.,* 1901, **37**, 52.

[2] Deycke, *Ges. Dtschr. Naturf. u. Arzte,* 1910.

[3] Bayon, Scientific Memoirs, Government of India, 1911, **42**, 1.

[4] Denney, U. S. Public Health Reports, 1934.

[5] Salle, *J. Infect. Dis.,* 1934, **54**, 347.

NaOH for one hour, then washed in sterile Ringer's solution before planting upon culture media; (3) small pieces of leprous tissue were submerged in 1% sterile trypsin solution for 24 hours at 37°C., after which the softened material was transferred to a variety of culture media; (4) smear preparations of the juice from macerated bits of the untreated fresh leprous tissue were stained by Ziehl-Neelsen's, Loeffler's, Gram's and Gabbet's methods, respectively; (5) bits of untreated fresh leprous tissue were planted upon a variety of nutrients including living chick embryo and growing tissue *in vitro*. Microscopic studies of the various materials were made every 3-5 days, over a period of approximately 6 months, which, in our hands, was the time required by *B. leprae* to become established in culture as a macroscopic growth.

Morphological features. The multiplying *B. leprae* of the initial culture shows only minor alterations in size, shape and arrangement of the chromatin from that observed for the bacillus in the human lesion. The leprosy "globi" which the author regards as intracellular colonizations, do not occur in culture even when the nutrient employed is living tissue. Such changes, however, as do occur are not as marked as those seen for the tubercle and diphtheria bacilli when cultured away from their natural habitat. In no sense are the morphological variations to be considered in the category of developmental "stages" in the life cycle of the organism. Furthermore, the slight pleomorphism of the Hansen organism is only noticeable during its period of adaptation to a purely saprophytic environment.

Under cultivation the leprosy bacillus early loses its sharply circumscribed dense bacillary masses known as "globi". In cultures that are established, these curious formations are never seen. The "globi" in tissue transplants become larger in size from week to week, due apparently to the increase in number of their contained bacilli. Later the "globi" lose their sharpness of outline when a separation and spread of the bacilli occurs into the surrounding medium. These changes to the "globi" are regarded as the forerunner or early sign of *in vitro* multiplication.

With regard to the individual bacilli, many become distinctly larger, less curved, more plump and rounded instead of pointed at the ends. Changes in the distribution of the chromatin also occur in that the so-called "beads" or "granules" in the bacilli become larger and more numerous. Certain rods have a sharply defined granule at either pole, while others possess only a large centrally located granule, and still others may have 4 to 6 granules. The bipolar arrangement of the granules accounts for the leprosy organ-

ism in stained preparation resembling certain forms of the diphtheria bacillus from culture. *B. leprae* at this period of its growth is distinctly diphtheroidal. In culture, the viable bacilli are always outlined by a cell-wall or membrane.

Before there is any definite evidence of macroscopic growth the transplanted bits of leprosy tissue contain great numbers of extracellular granules which are non-acid-fast though positive to Gram's method of staining. These extracellular granules are chromatin masses and are similar to the "Much" granules in the lesion of tuberculosis. While some writers regard them as "degeneration" forms, others believe that they may be analogous to certain spores. Germination of these "bodies" has not been observed, though carefully studied with this point in mind. The fact that these extracellular granules appear in great numbers in early cultures during the period of saprophytic adaptation supports the view that they do represent some sort of transitional stage of the organism. It is possible that these granules are the forerunner of the permanent bacillary forms that have adapted themselves to the new environment. In this connection it is noteworthy that when the leprosy bacillus has completely acquired the power to grow saprophytically, these "free" granules are not observed.

About the time the leprosy culture becomes visible to the naked eye, the bacilli appear plump, short and ovoid. Intracellular granules are conspicuous by their absence, because the chromatin is now homogeneously distributed. During the transitional period the bacilli show a well defined cell membrane. Still later, however, when the culture will grow upon ordinary laboratory media, the bacilli for the most part become again distinctly "beaded" with metachromatic granules.

Tinctorial Properties. The viable Hansen bacillus of leprosy whether *in vivo* or *in vitro* is always acid-fast in the generally accepted sense of the term. Its resistance to certain strengths of decolorizing agents is as constant as that of the tubercle bacillus; however, it will not resist decolorization to the same degree as will the latter. The Ziehl-Neelsen and other methods used for staining tubercle bacilli in tissue, will not satisfactorily demonstrate the leprosy organism under the same conditions. Likewise, these methods are unsuitable for discernment of *B. leprae* from culture, unless a weaker strength of the acid-alcohol decolorizer, or a shorter period of treatment is employed.

At no time during the period (6 months) in which *B. leprae* is adjusting itself to artificial growth conditions, was there noted

any difference in respect to its acid-fastness. Throughout the entire 6 months' period of microscopic growth, the same degree of resistance to decolorizing agents was observed for the bacilli in the transplanted leprous tissue as is the case of the organism in the human leproma. There is, however, far less resistance to decolorization during the transitional stage that just precedes the initial macroscopic growth. In the opinion of the author this fact may explain the statement of Salle that the subculture of *B. leprae* from Carrel's living tissue medium to ordinary nutrients, becomes non-acid-fast.

B. leprae is at all times strongly positive to Gram's method of staining. Where this method is employed without counter-staining, the individual bacilli simulate short chains of streptococci. This appearance is due apparently to the retention of the stain by the denser chromatin masses. Gram's method, more than any other, accentuates in "bas-relief" the chromatin granules. Differences in behavior of the leprosy bacilli to the alcohol treatment in the Gram's method have not been observed. In other words, the so-called involutions, transformations, degenerations and chromatin granules are equally positive in their reaction to this method of staining.

B. leprae when stained by Loeffler's methylene blue method for *B. diphtheriae* reveals the fact that the intra- and extracellular granules are distinctly metachromatic. Many of the stained bacilli from early cultures are striking in their morphological appearance to the bipolar forms of *B. diphtheriae*. Undoubtedly this would account for the diphtheroidal "stage" of the leprosy organism referred to by some writers.

Summary. The uncontaminated subcutaneous nodules from 4 cases of human leprosy were cultured upon a variety of media and under various conditions, in order to afford a systematic study of the morphology and tinctorial properties of *B. leprae* during its period of adaptation to an artificial environment. All changes occurring for the Hansen bacillus *in vitro* were noted and compared with the morphology and staining properties of the organism in the original human leprous lesion.

During the entire period of observation (6 months) only minor alterations in size and shape of *B. leprae* were noted, and these were no more than what occurs for the tubercle bacillus under similar conditions of cultivation. Where multiplication occurred in the transplanted leprous tissue the acid-fast rods became longer, thicker, less curved and more distinctly diphtheroidal in appearance,

because of the bipolar massing of the chromatin. So striking was this arrangement of the chromatin granules that with Gram's and Loeffler's methods of staining, certain of the *in vitro* growing forms are indistinguishable from the bipolar metachromatic granular types of *B. diphtheriae*. Later, when the organism is acquiring the power to grow independently of the host tissue it becomes shorter, more plump and often without granules. Still later, after macroscopic growth is well established, the bacilli assume the morphology of those in the transplanted leprous tissue. At no time were branching filamentous and interlacing streptothrical forms encountered.

The carbol fuchsin stained microorganism of leprosy, whether in culture or the living host tissue, is less resistant to decolorization by the ordinarily used agents for this purpose, than is the tubercle bacillus. Failure to realize this fact may account for the reports of non-acid-fast diphtheroidal and streptothrical forms of the Hansen organism. *B. leprae* in the human lesion is decidedly more resistant to decolorization than it is from macroscopic culture. Where the Ziehl-Neelsen method is employed there occur varying degrees of acid-fastness to complete decolorization of the bacilli from culture. In a given field of the stained preparation there will be rods that have only partially, and still others that have not, retained the stain. On the other hand, where the Ziehl-Neelsen method is used and the time-period for decolorization is greatly lessened, the leprosy bacilli will remain deeply stained.

The Hansen organism of human leprosy is an acid-fast, Gram-positive bacterium that possesses much the same morphology and staining properties as the tubercle bacillus.

Finally, in the experience of the author,[6] the macroscopic culture of *B. leprae* is obtained only after months of careful cultivation upon split-protein media, preferably that of the excised, transplanted autolyzing leproma. Once the organism has successfully passed through the transitional period of *in vitro* adaptation, growth takes place readily upon the ordinary laboratory nutrients.

[6] Duval, C. W., and Holt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 828.

7740 P

Absorption from the Rabbit's Colonic Spindle (Fusus Coli) and Adjacent Sections of the Colon.

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The colonic spindle of the rabbit is an anatomical and functional modification of the gut connecting the ascending colon with the descending colon.[1] One of its functional activities is to squeeze out the liquid contents of the scybala formed in the ascending colon and to deliver these scybala, now hard and relatively dry, to the immediately adjacent descending colon.[2]

The present paper deals with the rate of absorption occurring in (1) the colonic spindle, (2) the ascending colon proximal to the spindle and (3) the descending colon distal to the spindle. A 1% solution of strychnin sulphate was used to betray absorption, the dose being 1 mg. per kilo rabbit injected into the ligated section of the gut.

The rabbits were narcotized satisfactorily by injecting 5 mg. of morphin sulphate per kilo subcutaneously. After laparotomy, a section of the ascending colon (single row of haustra) or the spindle itself, or a section of the thin walled descending colon was doubly ligated, carefully sparing the blood supply. The appropriate dose of strychnin was then injected into the lumen of the empty ligated section and the abdomen closed with 2 rows of sutures. After release, the animal was tested by tapping at 2-5 minute intervals for the development of hypersensitiveness and opisthotonos.

The ligated sections were not of equal length, in order to equalize

TABLE I.

	Ascend. Colon (2-3 cm.)	Spindle (5 cm.)	Desc. Colon (5 cm.)
Number of rabbits	6 ♂	7 ♂, 1 ♀	4 ♂, 3 ♀
Hypersensitiveness	6 (in 3-9 min.)	8 (in 5-17 min.)	7 (in 10-17 min.)
Opisthothonos	6 (in 6-17 min.)	2 (in 10-13 min.)	0
Death	4 (in 8-25 min.)	0	0
Recovery	2	8	7

None of the female rabbits were pregnant.

[1] Auer, J., *J. Pharm. and Exp. Therap.*, 1925, **25**, 140; PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 301.

[2] Auer, J., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 331.

the absorptive areas; in the ascending colon 2 to 3 cm. lengths were ligated, while 5 cm. lengths were used in the other 2 sections. The absorptive area in the ascending colon was, therefore, if anything smaller than that in the spindle or the descending colon.

Either an autopsy or a biopsy was made on every animal to determine the condition of the ligated sections of the gut, the normality of the kidneys and of the animal in general.

The main results are shown in Table I.

The results indicate that efficient absorption of strychnin is best in the ascending colon, less in the spindle and least in the descending colon.

7741 P

A New Development in Histospectrography.*

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Histospectrography, as developed by Policard,[1] and by Gerlach and Gerlach,[2] is a method of examining the elements in tissues which consists of passing a high frequency spark through a predetermined area in a section of tissue and by means of the spectrograph analyzing the rays emitted. The spectrograms will contain the lines characteristic of the elements encountered by the spark in passing through the tissue. It has been pointed out by Policard[1] and Gerlach and Gerlach[2] that one of the greatest difficulties encountered is the selection of electrodes; not only do the characteristic lines of the major element of the electrodes appear on the spectrum, but also those of even small impurities in the metal. In the course of our experiments with the technique, a means was devised whereby the purity of the electrodes is rendered immaterial, and the choice of basic metal almost so. In fact, for all practical purposes, our spectrograms contain lines characteristic only of the tissue.

* Aided by grants from the Macy Foundation; an appropriation from a grant made by the Rockefeller Foundation to Washington University for research in science; the Cyrus M. Warren Fund of the American Academy of Arts and Sciences.

1 Policard, A., *Protoplasma*, 1933, **19**, 602.

2 Gerlach, W., and Gerlach, W., *Die chemische Emissions-spectralanalyse. II Teil Anwendung in Medizin, Chemie und Mineralogie.* 1933. Voss, Leipzig.

The tissue support assembly differs entirely from that employed by other investigators. Policard supports tissue sections for analysis on a metal plate, which forms the lower electrode of the spark gap, carried by a special mechanical stage to permit the selection of the area to be sparked while it is under observation with a microscope. The upper electrode is a metal point immediately above the tissue. Gerlach and Gerlach's apparatus differs from Policard's in that a glass plate is interposed between the specimen and the base plate. With both procedures the possible area of localization is of the order of 1 mm. in diameter and lines are photographed from one or both electrodes.

It seemed to us that this large area of localization rendered the name "histospectrography" purely academic and made unnecessary microscopic observation while sparking. Hence, we select the areas before sparking by removing them as 1 to 3 cmm. segments by means of a corneal trephine or small scissors. These small bits of (fresh) tissue are placed on the ends of short lengths of pyrex glass rod, 2 to 3 mm. in diameter, where they adhere and are allowed to dry partially. Such specimens are rapidly and easily prepared and with no more possibility of contamination from the metals of the instruments used than in the case of obtaining tissue slices.

We then profit by the fact that for sparks which are not too intense the spectral lines from metallic electrodes are emitted at or near the surfaces of the electrodes. We use ordinary steel electrodes about 1.2 cm. apart and screen off from the spectrograph slit all light except that from about 5 mm. at the center of the spark. The tissue bits are inserted on their pyrex rods into the central portion of the spark and burned. Only the longest of the test exposures of air, glass and electrodes alone show traces of iron. Consequently there is little possibility of electrode impurities being registered on the plate, and with routine examinations of tissue specimens even the spectral lines of iron from the electrodes can be regarded as being absent (especially as iron is nearly always present in variable amounts due to traces of blood). However, since it is a simple procedure to change electrodes it is advisable to use some other metal than any particular one sought, if only to be doubly safe.

If it seems preferable for any reason to keep sections of organs under continuous observation while the selected areas are being burned, this is easily accomplished with the same electrode and screen set-up. A glass plate with a 3 mm. hole in the center is used to support the section. The spark passes through the tissue and the opening in the glass plate. In many instances it is desirable to have,

for comparison, spectrograms of known solutions. These are obtained by soaking a small roll of ashless filter paper in the solution and introducing it into the center of the spark. Strips of epidermis may be cut and held in small pyrex glass tubes and burned in the same way.

The high frequency generator used in our experiments is similar in design to that employed by the previously mentioned authors. Certain modifications were necessary, however, before the apparatus was adaptable to our purposes. These and other technical details will be fully described later.

With the techniques described, using a Gaertner L 250 W quartz spectrograph, we have experienced no difficulty in obtaining strong lines of Ca, Mg, K, Na, Fe, Cu, and P in a large variety of tissues examined.

7742 C

Action of Parathyroid Hormone in Normal and Hypophysectomized Pigeons.*

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Current studies of this laboratory on the relation of the various anterior pituitary hormones to carbohydrate and calcium metabolism made it necessary to learn whether the parathyroid influences the blood calcium level in pigeons as it is known to do in certain other animals. Data on this latter point only are presented here. Collip[1] has shown that mammalian species show extraordinary differences in their response to the parathyroid hormone, and could demonstrate no effect of the hormone on non-laying hens. Concurrently with the present study Hutt[2] observed in a case of idiopathic hypoparathyroidism in which the normal serum calcium level was restored with parathormone. Macowan[3] found parathormone to have no effect on the blood calcium of moulting hens.

Sugar was determined by the Hagedorn-Jensen method, calcium

* Aided by a grant from the Carnegie Corporation of New York.

1 Collip, J. B., *Can. Med. Assn. J.*, 1931, **24**, 646.

2 Hutt, F. B., and Boyd, W. L., *Endocrin.*, 1935, in press.

3 Macowan, M., *Quart. J. Exp. Physiol.*, 1932, **21**, 383.

by the Collip and Clark method. Usually only 1.0 to 1.8 cc. serum was used for the calcium determination, and blood was taken from the wing vein. Parathormone (Lilly) was employed in intramuscular injections. Though no attempt was made to find the time after dosage at which the maximum effect of parathormone is obtained the data of Tables I and II make it clear that in adult, normal, hypophysectomized and in thyroidectomized pigeons the serum calcium is markedly and rapidly increased. Both males and females gave this response; the average increase being 18% in normal, 24% in hypophysectomized, and 30% in thyroidectomized birds.

TABLE I.
Effects of injections of parathormone (Lilly) on blood sugar (mg. per 100 cc.) and serum calcium (mg. per 100 cc.) of normal tippler pigeons.

	First control 8/22		Test 8 hours after injection of 10 units 8/23		Test 22 hours after further injection of 20 units 8/24		Later control 8/29	
Bird	Sugar	Ca	Sugar	Ca	Sugar	Ca	Sugar	Ca
♀ A503	219	9.8	217	11.9	283	11.9	217	10.1
♂ A526	214	10.2	263	12.8	303	10.6	214	10.2

TABLE II.
Other tests of effects of parathormone on normal, hypophysectomized, and thyroidectomized pigeons.

		Dosage		Test		Control	
Condition of pigeon	No. of birds	Hours before test	Units	Sugar	Ca	Sugar	Ca
Normal	2	19; 4	20; 10	231	15.3	194	12.6
Hypophysectomized	2	19; 4	20; 10	199	13.9	184	10.4
Thyroidectomized	2	19; 4	20; 10	197	13.4	183	10.4
Hypophysectomized	2	5.5	12	180	10.9	183	9.6
Thyroidectomized	2	5.5	12	179	12.4	181	9.4

An unexpected but apparently definite (22%) effect of the parathormone injections on the blood sugar level was obtained in 5 of 6 tests made on normal pigeons—and in normal pigeons only. Possibly something other than parathyroid hormone (but present in "parathormone") was responsible for this action on the blood sugar.

Summary. The parathyroid hormone (parathormone) effectively increases the serum calcium level in normal, hypophysectomized and in thyroidectomized pigeons. Pigeons are therefore to be added to the few species now known to be suitable for use in studies which seek to identify the particular anterior pituitary hormone which stimulates the parathyroids. The parathormone preparation used

contained something which led to increased blood sugar values in 5 of 6 tests made on normal pigeons.

7743 C

Response of Adult Rat Testes Sex Accessories and Adrenals to Injections of Prolactin.*

OSCAR RIDDLE, ERNEST L. LAHR, ROBERT W. BATES AND CLARENCE S. MORAN.

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The testes of mature doves[1, 2] and the ovaries of mature fowl[3, 4] quickly and strikingly diminish in size during dosage with prolactin. In the fowl the oviduct (uterus) simultaneously involutes. In all these cases follicle-stimulating hormone (F.S.H.), admixed with some thyreotropic hormone, has a directly opposite effect. It is of more than theoretical importance to know whether prolactin exercises a similar action in mammals, and for a first test of this point we chose the testes of adult rats. It seemed best to make simultaneously tests of effects of F.S.H. and Prolan (Elberfeld) on some of these adults; and also to search in several organs for size changes which might throw light on the action of each of the hormones used. A group of younger rats was ultimately included in the study.

Adverse effects upon the ovary have been repeatedly reported, and such effects upon the testis occasionally found, from the use of crude anterior pituitary preparations, and attempts have been made to associate such adverse effects with one or another pituitary principle. On this point Loeb[5] recently concluded that the hormone causing follicular atresia is probably closely associated or identical with the thyreotropic hormone. It is therefore noted here that the prolactin used was free or practically free of thyreotropic (and F.S.H.) hormone, and that thyreotropic hormone is found in fair quantity in our F. S. H. preparations (also in all such preparations made by others from pituitary tissue and examined by us).

* Aided by a grant from the Carnegie Corporation of New York.

1 Riddle, O., and Bates, R. W., *Endocrin.*, 1933, **17**, 689.

2 Riddle, O., Bates, R. W., and Lahr, E. L., unpublished data.

3 Bates, R. W., Lahr, E. L., and Riddle, O., *Anat. Rec.*, 1933, **57**, 30.

4 Bates, R. W., Lahr, E. L., and Riddle, O., *Am. J. Physiol.*, 1935, in press.

5 Aff, H. M., and Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 957.

TABLE I.

The Response of Testes, Sex Accessories, and Adrenals of the Rat to injections of Prolactin, F.S.H., and Prolan.

Injected or control	Age mo.	Dosage		No. of rats	Body wt. with change gm.	Wts. of untreated left, change in treated right		Organ wts. at end of treatment		
		Units or mg. daily	Duration days			Testes mg.	Epididymis mg.	Prostate + seminal vesicle mg.	Cowper's glands mg.	Adrenals mg.
Muscle extract	7	8 mg.	13-23	4	294+22	1574—28	585— 4	2008	54.8	48.0
Prolactin		10 B.U.	15-22	10	290+ 8	1591+20	601—18	2506*	39.9	57.8
F.S.H.		2.5 mg.	14-21	10	281+14	1620+85	592+52	2537	56.5	46.8
Prolan (Elb.)		30 R.U.	15-23	9	290+ 1	1541+ 2	574+29	2866	53.5	52.5
Muscle extract	8+	5 mg.	8	3	314— 1	1748—45	617— 2	2828	46.7	52.3
Prolactin		20 B.U.	8	3	327+ 4	1783+ 2	660+13	3425	42.1	64.2
F.S.H.		5 mg.	8	3	315—12	1780+35	625+73	3395	50.6	65.5
Prolan (Elb.)		90 R.U.	8	3	331—15	1737+16	658— 6	4417	74.0	59.8
Muscle extract	2.8	4 mg.	11	4	177+37	1306+150	275—13	1127	24.0	34.7
Prolactin		15 B.U.	12	4	177+30	1316+138	259+ 5	1166	20.1	39.9
F.S.H.		4 mg.	12	4	176+22	1286+ 98	286+73	1134	31.5	34.9

*Omitting an extremely large (5.3 grams) and abnormally hard prostate this average value is 2194 mg.

Before starting injections we removed and weighed the left testis and epididymis of each rat, and in the case of these 2 organs these initial weights serve as a basis for calculating gain or loss of weight by the right testis during injection. We injected a group of 11 rats, aged 1 year or more, obtained through the courtesy of Dr. L. B. Mendel and of Dr. H. C. Sherman, but this material proved too heterogeneous (3 deaths) to justify consideration here. Weights of thyroids, livers and pituitaries were obtained on all rats, but significant differences were not found and those data are not tabulated. The significant data are given in Table I.

Under prolactin dosage no reduction of size in either testes or epididymis is found. Histological differences between the control and treated testes are also not apparent. Perhaps equally remarkable is the slight or absent *stimulating* effect of a potent preparation of F.S.H. on the adult (not senile) rat testis—a result earlier reported by Smith[6] and by Evans *et al.*[7] In our tests, as in those of Moore and Price,[8] Prolan apparently did not increase the weight of testis and epididymis though it led to marked enlargement of the prostate and seminal vesicles. Testis size in the adult rat is apparently little affected by any pituitary hormone which has elsewhere been found to produce marked change in gonad size.

An effect of prolactin on weights of Cowper's glands and adrenals is indicated by these data—Cowper's glands undergoing size diminution and the adrenals an apparent enlargement. The weight difference between the adrenals of the 10 prolactin injected rats of our main (7 mo.) group and the values obtained for the other 23 rats of the group is 5 times its probable error and is probably significant. A different F.S.H. preparation (No. 243) was used, and in higher dosage, on the smallest group (8+ mo.) of rats. At that level of dosage prolactin is detectable in No. 243, and it is therefore possible that prolactin also increased adrenal weight in the F.S.H.-injected rats of that group.

From studies on secondary effects of prolactin in birds it seems probable that the hormone affects Cowper's glands secondarily through the testis. In the case of the adrenals, however, the effect is probably either a direct effect of prolactin, or through a stimulation of the pituitary to a release of adrenotropic principle, or it is

[6] Smith, P. E., Chap. 15, Sex and Internal Secretions. Williams and Wilkins, 1932.

[7] Evans, H. M., Meyer, K., and Simpson, M. E., *Mem. Univ. of Calif.*, 1933, **11**, 1.

[8] Moore, C. R., and Price, D., *Am. J. Physiol.*, 1931, **99**, 197.

a direct effect of a separate adrenotropic principle still contained in the prolactin (chiefly our No. 237) used in this study.

Summary. In adult (not senile) rats injected during 8 to 22 days with 10 or 20 units per day of prolactin the weights of testes were not diminished. By this dosage the weight of Cowper's glands are apparently decreased, and that of the adrenals increased. In relatively high dosage during similar periods follicle-stimulating hormone (+ some thyreotropic) and Prolan are found, confirming earlier work of others, to have almost negligible power to cause an increase of size in such testes. Prolan led to marked enlargement of the seminal vesicles and prostate, and in the heavy dosage used it was apparently adverse to body growth.

7744 C

Effect of Splenectomy and Other Operative Procedures on Platelets as Determined Volumetrically.

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An increase in the number of circulating thrombocytes has been observed following operations,[1, 2] parturition[1] and fractures,[3] but is greatest after splenectomy.[4, 5] This increase has been attributed to tissue injury[1] and in the case of splenectomy to additional factors.[6] The period of maximum increase coincides with that period in which post-operative thromboses are most likely to occur.

We have repeated the above observations using the volumetric method[7] of platelet determination, since this has proved to be more reliable in our experience than any method of counting and in addition indicates the amount of platelet substance present, rather than simply the number of platelets irrespective of their size. In normal subjects the platelets form from 0.4 to 0.6% of the whole blood volume.

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[1] Dawbarn, R. Y., Earlham, F., Evan, W. H., *J. Path. and Bact.*, 1928, **31**, 833.
[2] Cramer, W., Bannerman, R. G., *Lancet*, 1921, **1**, 1048.
[3] Galloway, J. F., *Lancet*, 1931, **1**, 1082.
[4] Evans, W. H., *J. Path. and Bact.*, 1928, **31**, 815.
[5] Shore, B. R., and Kreidel, K. V., *Ann. Surg.*, 1934, **99**, 307.
[6] Krumbhaar, E. B., *Am. J. Med. Sc.*, 1932, **184**, 215.
[7] Van Allen, C. M., *J. Lab. Clin. Med.*, 1926, **12**, 282.

TABLE I

Showing the platelet determination prior to operation, the highest reading and the day on which this occurred in each case. The case numbers correspond to those in Figure I.

Splenectomy.					Postoperative and Postpartum.				
Case No.	Disease	Platelets	Maximum rise		Case No.	Operation	Platelets	Maximum rise	
			Platelets	Day				Platelets	Day
1	Familial hemolytic icterus	.5	2.25	13	1	Bone tumor	.45	.75	4
2	” ” ”	.6	3.6	15	2	Thyroidectomy	.60	.62	3
3	” ” ”	.3	3.1	9	3	Fistula-in-ano	.72	.75	1
4	” ” ”	.26	2.6	14	4	Carcinoma of rectum	.93	.70	2
5	” ” ”	.6	2.3	13	5	Thyroidectomy	.72	.69	4
6	” ” ”	.3	1.53	10	6	Mastectomy	.41	.94	13
7	Banti’s syndrome	.45	2.27	10	7	Herniorrhaphy	.52	.91	3
8	” ”	1.1	1.99	11	8	”	.47	1.17	9
9	” ”	.16	1.93	19	9	Thyroidectomy	.44	.62	1
10	” ”	.4	1.3	10	10	Hemorrhoidectomy	.52	.85	13
11	” ” (luetic)	.2	1.8	15	11	Mastectomy	.48	.77	8
12	” ”	.1	1.3	14	12	Skin graft	.63	.78	10
13	” ”	.55	1.2	7	13	Exploratory laparotomy	.39	.75	9
14	Familial hemolytic icterus	.3	1.7	11	14	Appendectomy	.54	.73	6
15	Thrombocytopenic purpura	.28	1.44	12	15	Cholecystectomy	.57	.92	2
16	Sickle cell anemia	1.0	1.60	12	16	”	.47	.69	5
17	Felty’s syndrome	.15	.3	6	17	”	.44	.83	17
					18	”	.88	1.82	10
	Average	.31	1.89	12	19	Postpartum	.45	.50	5
					20	”	.45	.46	3
					21	”	.44	.73	6
					22	”	.53	.53	8
					23	”	.48	.85	9
					24	”	.43	.76	9
						Average	.54	.79	6

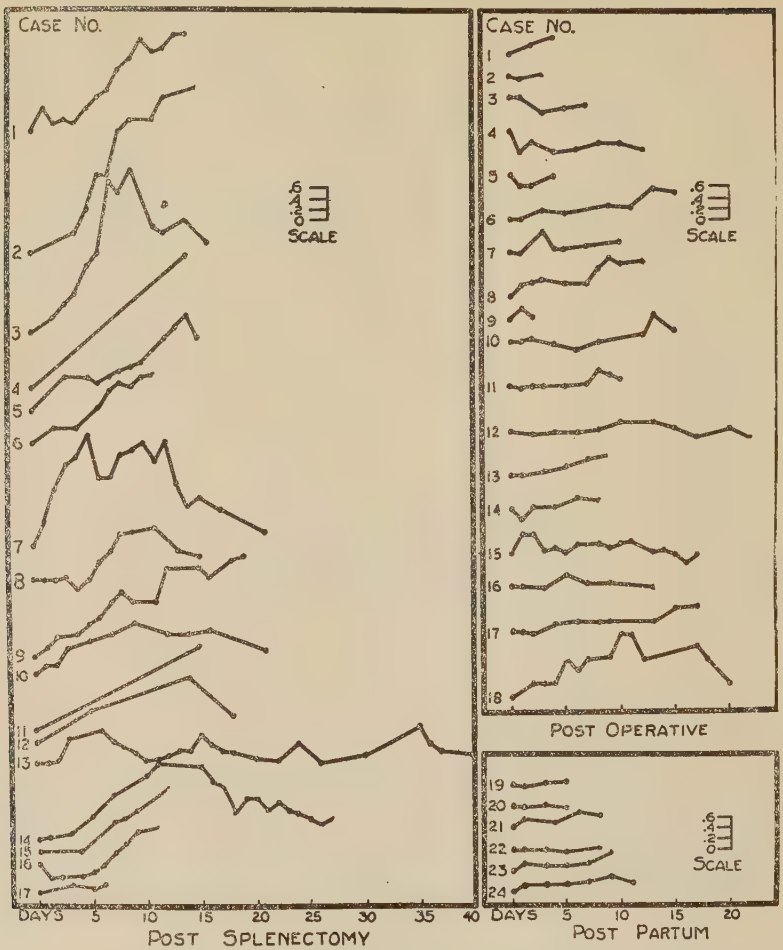

FIG. 1.
Platelets after operation.

Table I and Fig. 1 show that a variable but moderate increase in the platelets occurred following operations, while in the postpartum cases there was only a very slight rise. There was a striking increase after splenectomy although a considerable variation in the degree and rapidity of the rise was noted. One patient (Case 11) was followed intermittently for 3 years and at the end of this time the platelets were present in normal amounts. Only one splenectomized patient (Case 17) failed to show a noteworthy increase in platelets. This patient died of mesenteric thrombosis on the ninth post-operative day. Symptoms of this complication appeared on the seventh day while the last platelet determination, 24 hours previously, had shown no significant increase in these bodies.

These results correspond in general to those obtained by platelet counts and indicate that increase in the platelet volume is not the primary factor in post-operative thrombosis.

7745 C

Oestrin and Progestin Content of the Corpus Luteum of the Sow.

C. A. ELDEN. (Introduced by J. R. Murlin.)

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The oestrin and progestin content of the corpus luteum of the sow was determined at various stages of development. Ovaries were obtained fresh from the abattoir and separated into the following groups: 1, early corpora lutea up to about the sixth or seventh day from the time of rupture of the Graafian follicle; 2, fully formed corpora lutea representing the various stages of early and middle pregnancy; 3, degenerating corpora lutea, and 4, corpora albicans. This separation does not take into account the correlation of the development of the egg and embryo, since the large amounts of ovaries were primarily for the preparation of progestin. However this macroscopic separation demonstrated that there is variation in the level of progestin which has physiological significance. Due care was taken in the dissection of each batch of corpora lutea that follicular fluid was not a contaminant.

Progestin and oestrin were prepared and separated according to the method of Allen.[1] The progestin content was determined according to the method of standardization of Corner and Allen.[2] The oestrin level was obtained by the vaginal smear test using castrated female rats. The number of rabbit units of progestin and the number of rat units of oestrin per 100 gm. of corpora lutea are recorded in Table I.

It is to be noted that the progestin content is highest during the

TABLE I.
Rabbit Units of Progestin and Rat Units of Oestrin per 100 gm. Corpora Lutea.

Batch No.	1	2	3	4
Rb. U. Progestin	4.1	3.1	1.6	less than ¼
R. U. Oestrin	2.7	3.4	3.0	1.3

[1] Allen, W. M., *J. Biol. Chem.*, 1932, **48**, 591.

[2] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

first 6 to 7 days. If the hemorrhage weight were not considered in the above, the level of progestin would be still higher since the hemorrhage which occurs in about 50% of the corpora lutea at this stage[3] increases the weight of the tissue. This high content of progestin correlates well with the active growth of the granulosa cell during this period.[3] It can be physiologically explained by the fact that a completely proliferated endometrium is necessary for implantation of the fertilized ovum when it reaches the uterus.

The oestrin content of the corpus luteum is more or less uniform suggesting saturation of tissue and not actual preparation of oestrin. The values found here are about half those obtained by D'Amour *et al.*[4]

7746 C

Results of Repeated Determinations of the Blood-Cerebrospinal Fluid Barrier.

H. W. LOVELL AND J. R. BROWN. (Introduced by W. Malamud.)

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Investigations of the barrier between the blood and cerebrospinal fluid by the Walter method[1] and its modifications[2–5] have amply proved its value as an aid in the diagnosis and treatment of mental diseases. A number of variations from this generally accepted procedure have been suggested as the result of studies intended to show that the distribution of bromide between the blood and cerebrospinal fluid does not attain an equilibrium at the end of the 5-day period of bromide ingestion.[6] Recommendations have been made that would alter the method or even change it entirely.

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[3] Corner, G. W., *Am. J. Anat.*, 1919, **26**, 117.

[4] D'Amour, F. E., D'Amour, M. C., and Gustavson, R. G., *J. Pharm. and Exp. Therap.*, 1933, **49**, 141.

[1] Walter, F. K., *Z. f. d. ges. Neurol. u. Psychiat.*, 1925, **95**, 522.

[2] Hauptmann, A., *Z. f. d. ges. Neurol. u. Psychiat.*, 1926, **100**, 332.

[3] Malamud, W., and Mullins, B. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 871.

[4] Malamud, W., Mullins, B. M., and Brown, J. R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1084.

[5] Malamud, W., Brown, J. R., and Mullins, B. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 733.

[6] Fremont-Smith, F., Dailey, M. A., and Sloan, D. H., *Trans. Am. Neurol. Assn.*, in press.

The chief purpose of this study is to set forth the results obtained in a series of patients who received from 2 to 6 spinal punctures at 3-day intervals following the administration of varying amounts of bromide. The test employed, including the correction for the

TABLE I.
Bromide Content of Blood (Mg. per 100 cc.).

Patient	Days after starting bromide (Five-day bromide test)							
	6	9	12	15	18	19	21	22
1	81.5	84.7	80.2	76.1	75.7		73.2	
2	71.4	62.5	60.0*	54.7		50.8		44.8
3	65.2	67.3	55.5					
4	75.0	62.5	57.7	50.0		42.9		37.5
5	142.8†	147.8	121.0	90.9		76.9		65.2
6	150.0†	103.4	93.8	82.4				
7	100.0†	100.0	88.2	87.2*	86.2		84.7	
8	147.0†	132.5	97.4		81.5		67.3	
9	73.2	61.2						
10	68.8	61.7			48.4			
11	82.9	67.9						
12	65.8	55.5	50.0					
13	77.3	64.6	56.6	57.5	47.0*		47.0*	
14	87.7	90.4	78.9	70.4	68.2		60.0	
15	285.7‡	265.5	219.0*	193.6	174.4		150.0	
16	300.0‡	270.7	230.8	196.0	174.4		135.2	
17	68.2	54.5	58.8	57.0	50.0		50.0	
18	61.7	53.6	50.0		37.5			
19	72.1	62.5	50.0	50.0				
20	78.9	64.6	60.0					

*Corresponding cerebrospinal fluid specimens discarded; bloody.
†Patients previously receiving bromide; test bromide given.
‡Patients with bromide intoxication; test bromide not given.

TABLE II.
Permeability Quotients.

Patient	Days after starting bromide							
	6	9	12	15	18	19	21	22.
1	3.08	2.87	2.86	2.94	2.88		2.86	
2	3.09	3.13		2.70		3.28		2.85
3	2.72	2.39	2.78					
4	3.05	3.00	3.16	3.21		3.32		3.09
5	2.74	2.88	3.12	3.29		3.43		3.19
6	2.38	2.52	3.14	3.10				
7	2.32	2.38	2.45		2.73		2.76	
8	2.49	2.51	2.98		2.94		3.00	
9	2.86	2.71						
10	2.63	2.79			2.72			
11	3.15	3.00						
12	3.04	2.95	2.60					
13	2.73	2.46	2.60	2.79				
14	2.58	2.51	1.86	2.90	2.97		3.04	
15	2.02	1.95		1.85	1.91		2.07	
16	2.10	2.03	1.98	1.89	1.98		2.14	
17	2.92	2.91	2.64	3.13	3.04		2.96	
18	2.49	2.44	2.58		1.99			
19	2.08	2.51	2.60	2.66				
20	2.50	2.46	2.68					

difference between the chloride content of the blood and cerebrospinal fluid,[5] has been described.[3, 7] The data obtained are recorded in Tables I and II.

Patients 5, 6, 7, and 8 had ingested bromide before the study was made but not in sufficient quantity to mitigate their receiving the test dose. Patients 15 and 16 on admission to the hospital were toxic from prolonged bromide sedation and were therefore included in the series without receiving the test dose. The latter patients and experience with others suffice to vitiate the use of large doses of bromide, or, because of its cumulative effect, smaller doses given over a longer period of time. Toxic symptoms are not infrequently manifested when the bromide content of the blood reaches 150 mg. per 100 cc., and conversely, toxic patients as a rule do not show significant improvement until the blood bromide content has fallen to approximately 150 mg. Patients in whom the bromide content of the blood is unusually high (cases 15 and 16), have extremely low permeability quotients which, as long as the blood bromide is in excess of 150 mg. per 100 cc., vary insignificantly one from the other during a series of examinations. It is inferred therefore that a high concentration of bromide in the blood damages the blood-cerebrospinal fluid barrier permitting an excess of bromide to enter the cerebrospinal fluid, or that the cerebrospinal fluid is capable of absorbing a relatively high proportion of bromide from the blood which it releases almost proportionately with the blood until the blood concentration falls to approximately 150 mg. per 100 cc., when the cerebrospinal fluid increases disproportionately its release of bromide and thereby again makes possible significant variations in the permeability quotients obtained on successive examinations. The latter is substantiated by the fact that the average permeability quotient increases with the passage of time. The lower limit of drug dosage is governed by the technical unreliability of the colorimetric method when the bromide concentration falls below 50 mg. per 100 cc. of blood.

The average permeability quotient was calculated for each group of examinations made and the mean variation from the average quotient determined. The figures of 0.289 for the examinations made on the sixth day and 0.264 on the ninth day vary insignificantly, whereas those obtained on subsequent days vary widely except for that obtained on the sixth puncture. However, because the amount of bromide contained in the blood of test patients 18 or 19 days

[7] Malamud, W., Fuchs, D. M., and Malamud, N., *Arch. Neurol. and Psychiat.*, 1928, **20**, 780.

after discontinuing the drug is too low often for accurate study, the results obtained for this group are less reliable than those obtained on earlier punctures.

Individual permeability quotients obtained on successive punctures varied widely from their corresponding initial quotients except for those obtained on the second puncture or ninth day, Similarly the average variation of 0.127 for the second group from the initial average quotient was markedly less than that for subsequent groups. But 4 cases deviated in their quotients on the ninth day by more than 0.20 which is the probable error of the colorimetric method itself.[7] Since one of these varied only 0.21 we may consider that but 3 or 15% of the cases varied significantly from their initial quotients while 11 or 55% of the cases varied less than 0.10. Average and especially individual quotient variations after the ninth day were wide and inconsistent.

With a majority of 13 cases on the ninth day showing a negative variation from their initial quotients (and with the average quotient slightly though insignificantly lower), the inference is that the cerebrospinal fluid continues to absorb additional bromide following the first puncture. In some instances an increase in the blood bromide content on the ninth day is associated with an increased permeability quotient and *vice versa*. There is no significant correlation, however, and the variations are relatively small. Our deduction, then, is that at the end of the 5-day bromide ingestion period a state of equilibrium between the bromide of the blood and that of the cerebrospinal fluid has been reached which is maintained until the ninth day. After the ninth day the percentage of cases showing positive quotient variations, as well as wide individual variations, increases markedly which accounts for the gradual average quotient increase resulting from the relatively rapid release of bromide from the cerebrospinal fluid.

Summary. 1. Twenty patients were given repeated spinal punctures after ingesting varying amounts of bromide. 2. A state of equilibrium between the bromide of the blood and that of the cerebrospinal fluid is maintained from the end of the 5-day bromide ingestion period to the ninth day. 3. The permeability quotient tends to rise with the passage of time indicating that after the drug has been discontinued the return of bromide from the cerebrospinal fluid to the blood is relatively more rapid than the elimination of bromide from the blood. 4. Permeability quotients do not vary widely when the bromide concentration of the blood is high; significant variations are detected when the bromide content of the blood is

maintained between 50 and 150 mg. per 100 cc. 5. The method employed is practicable and provides an ideal concentration of bromide in the blood.

7747 P

Concentrated Fouadin in Treatment of Schistosomiasis Japonica in Rabbits.

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Khalil *et al.*[1] and Khalil[2] found that fouadin—antimony III-pyrocatechin-disulphate of sodium—was very efficacious against bilharziasis. Lee[3] and Lee and Chung,[4] however, did not obtain the same result in *Schistosomiasis japonica*. For treatment of this disease, a related compound of calcium salt called "Concentrated Fouadin", has been manufactured. Apart from its composition, the new drug is said to differ from fouadin in being more concentrated and less irritating. It contains 11% of antimony III-pyrocatechin-disulphate of sodium and calcium and is issued in ampoules. Each cc. of the solution has 14.3 mg. of Sb. III and 7 mg. of calcium.

To obtain experimental subjects, young rabbits of 1.5 to 2 kg. in weight were infected with the fork-tailed cercariae of *S. japonicum*. On the discovery of the ova in the feces, the animals were given 6 weekly intramuscular injections of Concentrated Fouadin until the eggs disappeared or died as determined by the hatching test. During treatment, particular attention was paid to the daily weights and to the appearance of toxic symptoms as a guide to the regulation of dosage. Thus it was found that the following scheme of administrations was well tolerated: an initial dose of 0.15 cc. followed by 0.25 cc. and 0.3 cc. for the second, third and subsequent injections respectively.

The effects of the drugs were judged from the findings at autopsy when the condition of the lungs, the liver and intestine was noted

[1] Khalil, M., Nazmi, M., Peter, F. M., El Din, M., Salach and El Betash, M. H., *Deutsch. med. Woch.*, 1929, **55**, 1125.

[2] Khalil, M., and Betasche, M. H., *Lancet*, 1930, **1**, 234.

[3] Lee, C. U., *Chinese Med. J.*, 1932, **46**, 1169.

[4] Lee, C. U., and Chung, H. L., *Chinese Med. J.*, 1933, **47**, 1411.

and the blood vessels in the lungs, the portal and mesenteric veins and inferior vena cava were searched for the presence of the worms. Tissues of the lungs, liver and intestine were also examined under a trichinia press to observe the condition of the ova.

Results. Of the 45 rabbits exposed to infection, only 27 were found suitable for the experiment, the results of which are given in Table I.

TABLE I.

		Amount of Concentrated Fouadin Used	Duration of Treatment (Days)	Killed (Days after Infection)
1. Cured	14	In 11 rabbits between 5.30-6.60 cc. Average: 6.20 cc. In 2, between 7-7.30 cc. In 1, 12.30 cc. in two courses	In 12 rabbits between 22-38 Average: 28.25 In 2, in 46 and 54 days respectively	From 99 to 268
2. Uncured	1	7.30 cc.	38	108
3. Died during treatment	5	1.20-6.00 cc.	5-28	Died: Majority between 58-75
	20			
Controls	7			Died: 3 in 96-98 days and 2 in 60 days Killed: 2 in 63 and 208 days respectively

Fourteen out of 20 animals were considered as cured because when killed after the controls had died of the infection, 9 had no worms and the ova in the tissues were not viable; 4 had only male worms and the ova in the organs were dead; one had parasites of both sexes but the females were few in number (4) and the eggs were dead.

One rabbit was pronounced uncured because 15 couples of worms which had normal gonads were found, and some living ova were seen in the liver and intestinal mucosa.

In connection with animals that were found free from the infection, it is to be noted that the total amount of Concentrated Fouadin used was not less than 6 cc. per animal and the duration of treatment was not less than 3 weeks.

Of the 5 which died, only one completed the course of injections which killed the flukes; the rest died during the treatment. Microscopically, the livers showed greater degree of generalized fatty

change than was seen in those of the control animals. Death, which took place at an earlier period than in the controls, may therefore be said to have been hastened by the action of the drug.

Five of the 7 controls died of schistosomiasis with cirrhosis of the liver in from 60 to 98 days, and 2 survived because the infection was light. All animals lost weight and were in poor health. It is to be remarked that in these animals, some ova (from 20 to 50%) were observed to die in the tissues even without any treatment.

7748 P

Possible Application of Chemical Reactions in the Determination of Pregnancy.*

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It is generally conceded that the reaction of the Ascheim-Zondek test depends upon the presence of a hormone in the urine of the pregnant woman. This hormone is believed to be either antuitrin, from the pituitary gland, or the so-called antuitrin-like hormone, from the placenta. Any chemical test that would be specific for either of these hormones would in all probability constitute a satisfactory test for pregnancy, which, if the test could be made simple enough, would have great advantages over the biological tests now in general use.

We have found that under carefully controlled conditions, that is, in a solution neutral to brom thymol blue, the commercial preparation of antuitrin will invariably reduce the oxidation-reduction dye, o-chlorophenol indolphenol, from blue to pink. The antuitrin-like hormone, commercial antuitrin S, will not cause this reduction, but both it and the antuitrin will reduce l-naphthol-2-sulfonate indophenol in an alkaline solution. These reactions can be obtained in water solution or with non-pregnant urines to which comparatively large amounts of the commercial preparations have been added. This test, simple and definite, gives promise of an excellent test for pregnancy.

In its practical application, however, it is necessary to extract the

* This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association.

very small amounts of the hormone present in the urine before applying the test since otherwise it may be masked by interfering substances. In the case of pathological urines, also, other proteins, unless previously removed, may give a similar reaction. It is in this extraction that we have encountered the greatest difficulties in the way of the method. It has not yet been worked out to a degree of accuracy that will permit the application of the test in a practical way. After trying, however, the various methods found in the literature for the extraction of these hormones, we have devised from them, with some variations introduced by us, a method of extraction that permits us to check the results of the Ascheim-Zondek test in a great majority of the cases.

At the present time we are using the following method: 13 cc. of urine, from the first specimen voided in the morning, collected without preservative, is precipitated with 1 cc. of 10% sodium tungstate, in a solution made acid to tetra brom phenolsulphonphthalein. After centrifuging down the precipitate, the liquid is decanted and discarded. The precipitate is shaken up with water, extracted with very dilute pyridine, and again centrifuged. The solution is decanted into acetone, made acid to the same dye, and the resulting precipitate centrifuged down. The acetone solution is discarded and the precipitate extracted with very dilute sodium hydroxide. The remaining precipitate is discarded and the dissolved portion poured into acetone, acidified for maximum re-precipitation, and then centrifuged. The final precipitate is dissolved in water, adjusted to neutrality with brom thymol blue, and the o-chlorophenol indophenol added dropwise. This dye, used in a 0.07% aqueous solution, deteriorates rapidly and should be prepared each week, but should stand several hours before using or be further diluted. Extracts from non-pregnant urines, without hormone, turn a distinct blue; those from pregnant urines, containing the hormone, fade to an almost colorless solution. While with very dilute urines, larger amounts must be used, ordinarily 13 cc. is sufficient. We have used this volume routinely since it is all that can be handled in 15 cc. conical centrifuge tubes, which fit the centrifuges available in most laboratories.

Using this technic we have tested 19 unknowns, specimens sent to us for the Ascheim-Zondek test. In 14 cases our results agreed with the Ascheim-Zondek findings. Of the remainder, 4 were false negative and one, false positive. Three of these negatives, dilute specimens, gave positive results when repeated with larger quantities of urine. Lack of material prevented a similar retest on the 4th. The

specimen giving the false positive caused an enlargement of the uterus and opening of the vagina of the test animal, but, in the absence of ripened follicle or corpus luteum, was read negative.

Seven specimens from normal non-pregnant women were run without an error, but one out of 4 which contained albumin gave a false positive. Using larger quantities of non-pregnant urines did not give positive results. Five specimens from known pregnant cases, ranging from the 2nd to the 9th month, gave one false negative. This gives a total of 31 correct findings in 35 cases, or 88.5% correct.

A similar test may be made by adding the 1-naphthol-2-sulphonate indolphenol to the final neutral water solution, then adding dilute sodium hydroxide, which will bring out the dark color in the negative samples, while the ones with the hormone remain colorless. Antuitrin S will give this latter test, but is not extracted by the above method satisfactorily, and unless present in unnaturally large amounts, as when deliberately added to the urine sample, will not be obtained in amounts sufficient for the test.

Since neither of these hormones has been completely isolated, the basis of the above described tests is circumstantial. Even so these tests constitute an advance, and may assist in the identification of the hormones. Meanwhile, we are continuing our study to perfect a method of extraction that will permit the practical application of these reactions to the determination of pregnancy.

7749 C

An "Extinction" Phenomenon on Stimulation of the Cerebral Cortex.*

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In the course of stimulation experiments on the motor cortex of the monkey an interesting phenomenon was met, which we wish to report.

The monkeys (macacus) were anesthetized with "Dial" and ether; in two experiments ether was used exclusively. The motor cortex was exposed for stimulation. The electrical stimulations, which were

* This investigation was aided by a grant from the Research Funds of the Yale University School of Medicine.

given for a few seconds, consisted of several patterns of various pulses, obtained from a thyratron stimulator after Schmitt,[1] 60 cycle alternating current or an ordinary inductorium. Both biphasic and half-rectified pulses were used. The durations of the various stimulatory periods and the intervals between them, though variable at will, were rigidly controlled by a mechanical device driven by a synchronous A.C. motor. Unipolar and bipolar stimulation, polarizable and non-polarizable electrodes were used. The responses of the contralateral musculature were recorded with the isotonic method on a smoked paper kymograph. The anesthesia, the temperature of the animal and the external conditions of the cortex were kept as constant as possible over long periods of time. The wave form of the various stimulations was checked with the cathode ray oscillograph.

Under all of these experimental conditions the stimulations of a single focus of the motor cortex result in very constant responses, provided the intervals between the successive stimulatory periods are long enough. Then the only variation of the responses is that described in a previous paper[2] as "waves" or intrinsic fluctuations in cortical excitability. Usually, if very strong stimuli are avoided, intervals of half a minute to one minute are sufficient to obviate any disturbing influence resulting in variation of response, apart from the "waves" just mentioned. When the intervals between the stimulatory periods are reduced to 6 seconds or less the well-known phenomenon of primary facilitation, a marked increase in succeeding responses, promptly appears.

When the interval between two stimulation periods (stimulation frequency 53 per second) *is taken at 13 seconds, leaving all other factors* (shape, frequency and intensity of the pulses and duration of the periods of cortical stimulation) *unchanged, the response to the second period is much smaller than that to the first, or it may be entirely absent. As soon as the interval between successive stimulations is augmented again to 27 seconds or more, the original size of the response promptly reappears.* In other words, the response to a stimulation of the motor cortex, which alone would be fully effective, is small or absent if this stimulation is applied about 13 seconds after another equal stimulation. Some extinguishing factor must, therefore, be operative in the motor cortex or in the motor mechanisms involved, 13 seconds after an effective stimulation.

[1] Schmitt, F. O., *Science*, 1932, **76**, 328.

[2] Brody, B. S., and Dusser de Barenne, J. G., *Arch. Neur. and Psychiat.*, 1932, **28**, 571.

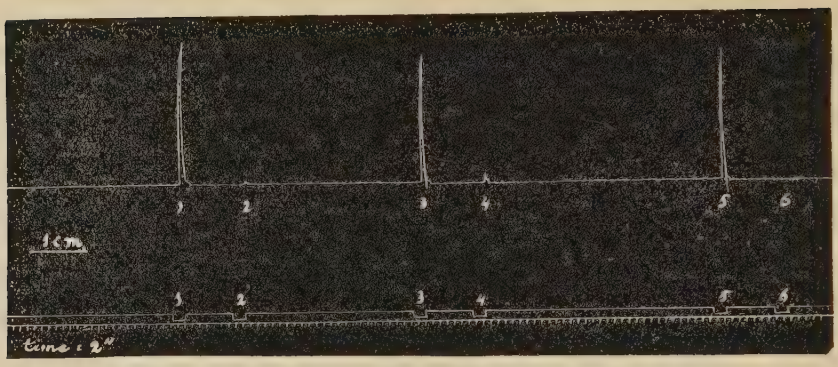

FIG. 1.

Exp. of Nov. 1, 1934. Monkey (macacus). "Dial" anesthesia. Three pairs of stimulation periods of one focus of left motor arm area, yielding extension of wrist: 1 and 2, 3 and 4, 5 and 6. Thyratron-tube discharges, resistance of 1000 ohms in series with animal. Duration, strength of stimulation, frequency and shape of pulses exactly the same in all 6 periods. Responses 2, 4, and 6 almost completely or completely extinguished because they are elicited 13 seconds after respectively antecedent stimulations. Stimulations 1, 3 and 5 fully effective, because of long preceding interval. Bottom line gives time in 2 seconds. Original size of graph may be determined by centimeter scale on left side.

Whether this *cortical extinction* or *inactivation* which appears upon stimulation of a single motor focus is partial or total depends largely upon the strength of the stimulations. With strong stimuli the phenomenon does not occur or is minimal; with moderate stimuli it is obvious; with just supraliminal stimuli it is almost complete or total. It looks as if in the production of this extinction two antagonistic factors were effective: the one, excitation of the focus by the applied stimulus, the other, the extinguishing factor, active at about 13 seconds after the last antecedent stimulation. The time required for the recovery from the extinction set by the first cortical stimulation seems to be a function of the frequency of stimulation. For frequencies around 50 pulses per second it is found to be optimal at an interval of 13 seconds, for frequencies around 100, around 20 seconds.

This extinction phenomenon has been observed in all monkeys under observation—whether in deep or light anesthesia—since it was first noted, and at every focus tested of all the 3 major subdivisions (face, arm and leg) of the motor cortex (areas 4 and 6 of Brodmann).

The fact that extinction is greatest with slightly supraliminal stimulation is important, as it indicates that the phenomenon cannot be explained on the basis of fatigue or exhaustion of the cortex. If this were so, one would expect to find it more obvious with stronger

stimuli and less evident with threshold stimuli. As has been stated, the reverse is true. The fact that decrease of the intervals between successive stimulations far within the duration of the optimal extinction interval, and, therefore, much closer to the refractory period, results in facilitation, is important, as it indicates that the phenomenon cannot be explained on the basis of a refractory period of the cortex following stimulation.

Extinction, as described here, is not to be confused with what is called cortical inhibition, which is cessation of a muscular response to stimulation of one focus of the motor cortex when a second, and antagonistic, cortical focus is concomitantly stimulated. Extinction occurs on stimulation of one motor focus following a preceding stimulation of the same focus, and that after the remarkably long interval of 13 to 20 seconds.

In conclusion it should be pointed out that this extinction is a physiological phenomenon, due to a functional change of the motor cortex under investigation or the motor mechanisms involved, and that it cannot be explained by physical inequalities of the various stimulations, because appreciable changes in polarization, resistance or impedance of the cortex are not responsible for the phenomenon. This has been shown by a new method of determining and recording of these various factors during electrical stimulation in living tissues, which will be published in the near future.

7750 C

Specific Factor in H. Pertussis Filtrate and Centrifugate.

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We described[1] a substance found in cultures of veal brain broth medium during the growth of *H. pertussis*. Subsequently,[2] further experiments with this material were reported. The present experiments were done (1) to ascertain the specific element in the filtrate, and (2) to obtain the sticky material that is produced in these cultures in more concentrated form.

[1] Toomey, John A., and McClelland, Joseph E., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 34.

[2] Toomey, John A., McClelland, Joseph E., and Lieder, Louis E., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 403.

After varied experimentation, I found that 4 fractions could be obtained in the following manner: *Fraction I, termed the acid precipitation fraction.* The filtrate obtained as previously described was brought up to pH 4.0 with acetic acid and then left in the icebox overnight. The bulky dark brown precipitate which formed was centrifuged off and was found to be slightly soluble in water or physiological saline. The remaining filtrate was adjusted to pH 8.0 with 25% NaOH and then back to pH 4.0 with acetic acid and put in the icebox overnight. The small precipitate formed was added to the previous one mentioned. Usually all the acid fraction came down after 2 such procedures, but occasionally a small amount could be obtained by a third precipitation. The combined precipitates formed the acid fraction of the filtrate.

Fraction II, termed the alkaline alcohol precipitate. The filtrate remaining after the previous fractionation was brought up to pH 8.0 with 25% NaOH; 2 volumes of 95% alcohol were added and the solution was put in the icebox over night. A heavy precipitate was obtained which was soapy white in appearance and easily soluble in water or physiological saline. The same procedure was repeated once or twice, each time the pH of the remaining filtrate being brought up to 8.0, and 2 volumes of alcohol added. One or 2 such procedures usually brought down all of this fraction. The combined precipitate was the alkaline alcohol fraction of the filtrate.

Fraction III, termed the acid alcohol precipitate. The pH of the filtrate remaining after the previous operation was brought up to 4.0 with acetic acid, 2 volumes of 95% alcohol were added and the solution was kept in the icebox over night. An adherent, sticky, brown precipitate was found which easily dissolved in water or saline. This fractionating procedure was repeated 2 or 3 times, all the precipitates were added together and termed the acid alcohol fraction.

Fraction IV, contained in the residual filtrate. Because of the large alcoholic content, this remaining filtrate was distilled to the original volume of the filtrate and then dried to a powder *in vacuo* or in an oven. This fraction was the residual fraction of the filtrate.

Fractions I, II, III and IV were standardized in physiological saline solution so that 1 cc. of the respective solution or solution suspension contained 4 mg. of the respective fraction. Eight rabbits were injected, each one of the fractions being used in 2 animals. Four separate injections were made at intervals of 4 days, the dosages being 0.5 cc., 1.0 cc., 1.5 cc., and 2.0 cc., respectively. The rabbits were bled 8 days after the last injection. The serum ob-

tained was used to agglutinate organisms, both old and recently isolated. Fraction I, the acid precipitate of the filtrate produced the highest specific agglutinin titer demonstrable by tests with straight and cross agglutinations.

Fractions II and IV produced no agglutinins, while Fraction III, the acid alcohol precipitate, caused the production of a negligible amount of specific agglutinins. Fraction I was not entirely pure, since it contained the acid precipitate that could be obtained from normal controls. The latter, however, when tested, showed no characteristics of specificity.

A great deal of work has been done recently with freshly isolated whooping cough strains grown on blood agar. Vaccines prepared from such cultures are being used in an attempt to prevent or modify the course of the disease. Endo-antigens have also been made from this type of culture. Clinically, the mere presence of *H. pertussis* organisms in the human does not cause a whoop *per se,* since they produce only the symptoms of a cold during the first week or so of the disease. The whoop that appears from 7 to 14 days after the onset of the infection is due to the thick, stringy, adhesive material that is produced by *H. pertussis* as the patient progresses to the later stages of the disease. Freshly isolated organisms do not produce a sticky exudate on placental or any other blood agar, but we have been able to produce material in the artificial media we have previously[1] described in the form of a pearly white, sticky mass, its physical characteristics being the same as those that are seen in the sputum of the human after a coughing spell. This stringy exudate does not consist merely of an increased number of organisms, as can be seen from microscopic examination. The physical characteristics of the growth which appears when these organisms are planted on solid medium (veal brain agar), suggest that this exudate is a product of *H. pertussis* metabolism. It adheres to the media so tenaciously that heavy wire has to be used to scrape it free after it has been growing for 4 or 5 days. It does not, however, penetrate nor break down the medium as some growing organisms do. Just a few surface tension observations with a duNoüy instrument show a cohesiveness of the exudate almost equal to thin glue. It resembles the covering of a Zuckerguss spleen or liver. Most of this stringy exudate has been ignored in the *B. pertussis* filtrate fractionation processes described previously.

H. pertussis growths in liquid cultures were centrifuged at high speed for from 30 to 60 minutes. All of the sticky masses at the bottom of the tubes were combined in one centrifuge tube, were

shaken and washed by repeated centrifugations in physiological saline until the supernatant fluid was clear. The latter was poured off and the remaining material weighed. A few drops of 25% NaOH were then added to the centrifugate and the material was arbitrarily standardized in saline so that 1 cc. contained a certain number of milligrams of wet weighed material, the amount depending upon the experiment. The entire centrifugate was ground in a ball mill for from 2 to 3 hours, and its pH brought up to 7.2; it was then bottled. The resulting mixture was not a true solution, but a finely precipitated substance which had the appearance of ground glass. When 0.1 cc. of a 1/100 dilution of this substance was injected into human subjects, it gave a localized inflammatory reaction that started in 6 and reached its maximum in 24 hours (50 individuals in all). When a 1/100 dilution was boiled for from 10 to 15 minutes, it lost its power to cause a reaction in human subjects (10 observations).

When this same dilution was concentrated to a powder *in vacuo* it lost from 90 to 95% of its wet weight (averages from 6 specimens). For example, the amount obtained from 250 cc. of culture media was 300 mg. of dried powder. The wet weight of the centrifugate in this case was 4.375 gm.

When this powder was injected intravenously into rabbits in the manner and doses described previously, it produced agglutinins specific for old strains of *H. pertussis*.

7751 C

The Effect of Gonadotropic Hormones During Gestation and Lactation.

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Many authors have studied the effect of gonadotropic extracts, prepared from pregnancy urine or pituitary tissue, on the course of gestation, and it is well known now that small doses do not interfere with the development of the embryos, but large doses cause abortion or intra-uterine fetal death.[1-4]

Our previous experiments[5, 6] convinced us that the development

[1] Katzman, P. A., Levin, L., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 873.

and maintenance of the corpus luteum of gestation cannot entirely be explained by the action of the known gonadotropic hormones in themselves, and that some other factor must be present during this period in order to produce the typical pregnancy ovary. It seemed of interest, therefore, to compare the reaction of the ovary to gonadotropic hormones during pregnancy with that obtained in the non-pregnant condition. We have, therefore, performed the following experiments:

Eight pregnant and 6 non-pregnant adult female rats received daily 20 rat units of the anterior pituitary-like hormone of pregnancy urine (A.P.L.) on 4 consecutive days by subcutaneous injection. They were killed on the fifth day. The ovaries of the non-pregnant animals were only slightly enlarged, averaging 80 mg. They contained numerous relatively small corpora lutea, and there was little luteinization of theca cells. The ovaries of the pregnant animals, on the other hand, were larger—averaging 113 mg. They showed marked signs of theca luteinization and contained numerous unusually large cystic corpora lutea. This atypical ovarian reaction to A.P.L. was still more marked in a second series in which 8 pregnant and 4 non-pregnant rats were given 50 units of A.P.L. over a period of 5 days. These animals were sacrificed on the sixth day. While the ovaries of the pregnant animals averaged 191 mg., those of the non-pregnant controls averaged 113 mg. Here again the presence of unusually large cystic corpora lutea, and the marked luteinization of theca cells distinguished the ovaries of the pregnant animals from those of the controls.

In another series of 6 hypophysectomized pregnant rats treated with 20 units of A.P.L. daily for 5 days and killed on the sixth day, we saw no increase in the weight of the ovaries as compared with uninjected pregnant controls (average 77 mg.). There was no follicle maturation and no corpus luteum formation, the only result of the treatment being a luteinization of theca cells as described in previous publications on non-pregnant rats treated with A.P.L after hypophysectomy.[7, 8]

From these experiments we conclude that during pregnancy

[2] Bourg, R., *Compt. rend. Soc. de biol.*, 1931, **108**, 216.

[3] Martins, T., and Fabiao, M., *Compt. rend. Soc. de biol.*, 1930, **105**, 791.

[4] D'Amour, F. E., D'Amour, M. C., and Gustavson, R. G., *J. Pharmacol.*, 1933, **49**, 146.

[5] Selye, H., Collip, J. B., and Thomson, D. L., *Anat. Rec.*, 1934, **58**, 139.

[6] Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 488.

[7] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

[8] Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 262.

A.P.L. leads to an unusually marked ovarian reaction with the formation of very large corpora lutea, and that this change in the effect of A.P.L. is conditioned by a change in the function of the hypophysis and not directly by the developing ova.

In this connection we should like to mention another experiment in which a gonadotropic extract, prepared from pig pituitary glands, was given to 6 pregnant and 6 non-pregnant adult female rats daily for 4 days. The dosage was approximately comparable to that of the urinary preparation used in the above experiment. The animals were killed on the fifth day. While the ovaries of the pregnant rats averaged 152 mg., showing again large cystic corpora lutea, those of the controls averaged only 91 mg. and consisted mainly of relatively small corpora.

Since the nervous stimulus of suckling is also capable of modifying the structure and function of the ovary,[9, 10] we have also studied the effect of gonadotropic hormones on lactating rats. Twenty-four animals, 12 lactating and 12 non-lactating adult controls, were used. Half of each group received 50 units of A.P.L. daily for 9 days, while the other half were treated for the same length of time with a comparable dosage of the gonadotropic extract prepared from pig pituitary. The macroscopical appearance of the ovaries and the increase in their weight (average 95 mg.) were very nearly the same in the 4 groups. Upon histological examination, however, we saw marked signs of thecal luteinization as well as granulosa luteinization in the ovaries of the A.P.L. treated lactating rats. The thecal luteinization in this group, just as in those previously mentioned, was particularly striking in the so-called "theca-nests"; that is, in those groups of theca cells that persist after the rest of the follicle becomes atretic. These theca-nests show no signs of luteinization in the A.P.L. treated, non-lactating animals or in any of these rats—lactating or non-lactating—which received the pituitary extract.

From these experiments we conclude that the effect of A.P.L on the ovary is modified during lactation, while that of the pituitary gonadotropic extract is the same in the lactating and in the non-lactating animal.

It seems, therefore, that the ovary of the lactating rat, like that of the hypophysectomized animal, shows a qualitatively different reaction to the gonadotropic preparations obtained from pregnancy urine and those prepared from the pituitary gland.

[9] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinol.*, 1934, **18**, 237.

[10] Selye, H., and McKeown, T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 683.

Although in the 4 groups of this last experiment the increase in ovarian weight was approximately the same, the appearance of the uterus was quite different in the lactating and non-lactating animals. While both the urinary and the pituitary extracts led to a great increase in the thickness of the uterus in the non-lactating animals, neither of these preparations had any marked effect on the thickness of the organ during lactation. Histologically the uterus of the non-lactating animals showed a marked increase in the connective tissue fibers of the mucosa. The epithelium was ciliated and its free surface was very irregular; mitotic divisions were rarely observed. The appearance of the organ is similar to that seen in the sterile horn in the case of unilateral pregnancy and may therefore justly be described as "progestational". The usual progestational proliferation was not produced by either the urinary or the pituitary preparation in the lactating animals, although the increase in ovarian weight was not markedly interfered with by lactation. In the lactating animals the uterine connective tissue fibers were poorly developed and the stroma of the mucosa contained more cells and fewer fibers. The epithelium was not ciliated and had a very regular free surface. Mitotic divisions of the epithelial cells were frequent. The general appearance of the organ was similar to that of the uterus of the untreated lactating rat. This inhibition of the effect of gonadotropic preparations on the uterus during lactation is probably largely independent of the ovarian response.

If one considers the thecal reaction obtained after A.P.L. administration during lactation as a result of an inhibition of hypophyseal function by nursing, it is remarkable that the weight increase of the ovary after the administration of this extract is not more distinctly inhibited. We thought that the administration of too large doses of A.P.L. might be responsible for this lack of inhibition in our animals.

We have, therefore, repeated these experiments with smaller doses. Our animals were divided into 3 groups. The first contained 6 lactating females; the second, 6 females weaned on the day of delivery. Injections were started in these 2 groups on the second day after delivery. In the third group we had 6 normal cyclic adult females. Twenty units of A.P.L. were given daily for 4 days and the animals were sacrificed on the fifth day. The weight of the ovaries averaged 85 mg. in the first group, 114 mg. in the second, and 59 mg. in the third.

This experiment shows that if adequately small doses of A.P.L. are given one may demonstrate an inhibiting effect of nursing on the

action of this hormone on ovarian weight. It shows, furthermore, that the unusually marked responsiveness of the ovary to A.P.L. which we observed during the course of pregnancy is not lost at parturition, and is still demonstrable during the first days post-partum if nursing is not allowed. A study of the uteri of these animals confirmed the findings reported above.

Summary. 1. Both the gonadotropic hormone of pregnancy urine and that prepared from pituitary tissue lead to a more marked ovarian response in the pregnant than in the non-pregnant rat. This increased responsiveness to gonadotropic hormones continues for some time post-partum if nursing is not allowed. 2. In hypophysectomized rats urinary preparations lead only to thecal luteinization, even when given during gestation. 3. Similarly in lactating and pregnant rats, the urinary preparation leads to thecal luteinization, but granulosa luteinization also occurs at the same time. Pituitary preparations do not lead to thecal luteinization during lactation. 4. Neither the urinary nor the pituitary preparation is able to produce the usual uterine reaction when given during lactation.

7752 P

A Crystalline Iron Chloride Molecular Compound of Urobilin and Stercobilin.*

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Evidence was presented previously[1] for the identity of crystalline urobilin and stercobilin as isolated from human urine[2] and feces,[3] respectively. These crystalline substances unfortunately do not have sharp melting points, nor is the "urobilin" absorption spectrum specific,[4] hence it was desirable to provide further means of positive identification, particularly for the study of substances which may be

* Aided by a grant from the research fund of the Graduate School of the University of Minnesota.

1 Watson, C. J., *Z. Physiol. Chem.*, 1933, **221**, 145.

2 Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1207.

3 Watson, C. J., *Z. Physiol. Chem.*, 1932, **208**, 101.

4 Fischer, H., *Ueber Blut, Blatt, u. Gallenfarbstoff. Oppenheimers Handb. d. Biochem. des Menschen u. d. Tiere. II Auflage. Ergänzungsband.* G. Fisher, Jena, 1930.

isolated subsequently. In this regard, Heilmeyer and Krebs[5] have recently mentioned certain differences in a stercobilin which they isolated by means of the procedure described by the writer. This question will be discussed in a later publication.

It has long been known that urobilin is capable of forming a number of metallic compounds. Heretofore, none of these have been isolated. A crystalline iron chloride molecular compound of mesobilirubin was described by H. Fischer, Baumgartner and Hess.[6] The corresponding compound of mesobilirubinogen is amorphous.[7]

In the present investigation it has been found that both urobilin and stercobilin yield a beautifully crystalline molecular compound with iron chloride. This crystallizes out of hot 25% hydrochloric acid in a yield of approximately 75%. Crystallization is best brought about by adding a hot, concentrated solution of $FeCl_3$ in 25% HCl, drop by drop to a tube containing the crystalline urobilin or stercobilin dissolved in hot 25% HCl, the reaction being carried out in a hot water bath with constant stirring. Immediately upon the addition of the $FeCl_3$ solution, the molecular compound comes out in an amorphous condition, but dissolves on stirring and further heating. Crystallization often commences while the solution is still hot, the crystals becoming more plentiful on cooling. They possess a glittering red-brown color. The individual crystals are tabular in habit, having a characteristic parallelopiped shape and an orange red color. The crystals obtained from the hydrochloride of urine urobilin are identical with those of stercobilin hydrochloride from the feces.

The substance is readily soluble in water, undergoing dissociation. After extraction of this water solution with chloroform, crystalline stercobilin hydrochloride may be isolated readily, indicating that the iron chloride has caused no change in the stercobilin molecule. Crystals which are allowed to stand in the air in a drop of the mother liquor eventually deliquesce. This is undoubtedly due to dissociation in the water remaining after the hydrochloric acid has in part volatilized. The molecular compound in alcoholic zinc acetate solution exhibits an intense green fluorescence. It is possible, however, that dissociation occurs in alcohol as well as water.

The preparation of this new iron chloride compound provides an added method of identification of crystalline urobilin or stercobilin. Since it is a method which is at once simple, and adaptable to as little as 2 mg. of material, it should be carried out wherever the nature of the substance isolated is at all doubtful.

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[5] Heilmeyer, L., and Krebs, W., *Z. Physiol. Chem.*, 1934, **228**, 33.

[6] Fischer, H., Baumgartner, H., and Hess, R., *Z. Physiol. Chem.*, 1932, **206**, 201.

[7] Fischer, H., and Niemann, G., *Z. Physiol. Chem.*, 1924, **138**, 293.

Elementary analyses as well as crystallographic measurements will be reported in detail in a later publication.

7753 C

Acute Agranulocytosis of Kala-Azar: Negative Effect of Urea Stibamine and Neostibosan on Blood of Normal Rabbits.

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Acute agranulocytosis, an important complication of kala-azar, has been observed in 8 of 71 patients suffering from visceral leishmaniasis.[1, 2] Six of these 8 patients exhibited a sudden and marked drop in the granulocytes at some time during treatment for kala-azar with either urea stibamine or neostibosan. Both drugs contain the benzene ring in their complex molecule. Acute agranulocytosis following the administration of certain compounds containing the benzene ring has been reported.[3] The following experiment was made in order to observe the effect of intravenous injection of urea stibamine and neostibosan on the blood of normal rabbits.

Eight normal rabbits were taken from the stock without selection. After a control period during which regular blood counts were made, 3 of the rabbits were given intravenous injections of urea stibamine, 3 others were given neostibosan and the remaining 2 were held as controls. An appropriate amount of either drug was dissolved in sufficient volume of double distilled water to make a 5% solution. The injection was given daily, except Sundays, through the marginal vein of the ear. Since urea stibamine is believed to be more toxic than neostibosan, the former was given in smaller individual doses. The total amounts were the same in all except in one (rabbit 1) in which it was double that of the others. Nothing was given to the control animals.

Counts were made on the blood obtained from the tributaries of the marginal vein of the opposite ear. These counts were done twice weekly and they included hemoglobin estimation, enumeration of total red and white blood cells and differential count of the leucocytes by the supravital method.

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[1] Zia, L. S., and Forkner, C. E., *Am. J. Med. Sci.*, in press.

[2] Zia, L. S., and Forkner, C. E., Trans. F. E. A. T. M. Ninth Congress, Nanking, China, 1934, in press.

[3] Madison, F. W., and Squier, T. L., *J. Am. Med. Assn.*, 1934, **102**, 1213.

TABLE I.

Negative Effect of Urea Stibamine and Neostibosan on White Blood Cells in Rabbits.

Rabbit No.	Drug given	Total dose	Duration of Experiment	Total W.B.C. per cumm.				Total P.M.N. per cumm.			
				Before inj.		After inj.		Before inj.		After inj.	
				Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
		gm.	days								
1	Neostibosan	4.79	65	18,100	6,100	15,150	5,200	10,220	3,782	10,756	2,028
2	,,	2.40	65	9,400	5,080	11,450	6,150	4,165	3,125	8,696	3,634
3	,,	2.40	65	28,000	6,100	24,000	6,000	9,526	5,022	15,277	3,151
4	None (Control)	—	65	11,700	4,750	8,400	6,000	6,515	2,277	6,048	2,948
5	,, ,,	—	54	11,750	11,750	12,800	7,350	—	—	7,710	3,825
6	Urea Stibamine	2.40	60	9,050	6,950	11,600	6,400	—	—	7,100	1,700
7	,, ,,	1.90	49	10,400	8,500	13,250	6,500	—	—	6,655	2,730
14	,, ,,	2.40	52	5,600	5,600	10,400	5,150	2,576	2,576	4,070	1,323

TABLE II.

Effect of Urea Stibamine and Neostibosan on the Hematopoietic Organs of Rabbits.

Rabbit No.	Drug given	Total dose	Duration of experiment	Histologic Findings				
				Spleen	Liver	Bone-Marrow	Lymph-nodes	
							Peripheral	Mesenteric
		gm.	days					
1	Neostibosan	4.79	65	Hyperplasia	Normal	Normal	Normal	Hyperplasia
2	,,	2.40	65	,,	,,	,,	Hyperplasia	
3	,,	2.40	65	,,	,,	Hyperplasia	Moderate hyperplasia	
4	None (Control)	—	65	Normal	,,	Normal	Normal	
5	,, ,,	—	54	,,	,,	,,	,,	
6	Urea Stibamine	2.40	60	Slight hyperplasia	,,	Slight hyperplasia	Hyperplasia	
7	,, ,,	1.90	49	Necrosis	Moderate cirrhosis, chiefly portal	Marked hyperplasia	,,	
14	,, ,,	2.40	52	Increased fibrosis and moderate hyperplasia	Extensive cirrhosis with proliferation of bile capillaries	Marked hyperplasia	Normal	

After the rabbits had received the prescribed amount of the drug, they were killed by intravenous injection of air. The liver, spleen, lymph-nodes and bone marrow were removed and immediately fixed in Zenker-formol solution (10% formalin). The sections were stained with hematoxylin and eosin. The results of the blood counts and histologic examination of the organs are shown in the accompanying tables. There was no appreciable effect on the body temperature or body weight as compared with the control animals.

It is to be noted that although the amount of either urea stibamine or neostibosan given to these experimental animals is far in excess of the usual amount given to patients for the treatment of kala-azar, yet neither the total white blood cells nor the granulocytes showed any significant decrease throughout the period of observation (Table I). On the other hand in these animals, there was hyperplasia of the spleen, lymph-nodes and bone marrow (Table II). In rabbits 7 and 14, there was cirrhosis of the liver and in rabbit 14 there was proliferation of the bile capillaries. Neither of the control animals presented similar changes. It should be pointed out that inasmuch as the controls did not receive the solvent of these drugs, this part of the experiment was not adequately controlled. Nevertheless, from the results obtained, it is concluded that repeated injections into normal rabbits of large amounts of urea stibamine and neostibosan did not produce in their peripheral blood anything resembling acute agranulocytosis.

7754 P

Blood Diastase as an Indicator of Liver Function.

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The method developed in this laboratory for the estimation of diastase in biological material satisfies 2 essential requirements: it yields accurate quantitative results, and is sufficiently sensitive to permit the determination of very small quantities of the enzyme.

The quantity of the enzyme we express as the amount of reducing matter, in terms of glucose, which is produced by a known amount of the enzyme-bearing material under standardized conditions. As applied to blood, when we state that the diastase value of human blood serum is on an average 120, this means that 100 cc.

of serum, incubated with 1.5% starch paste for ½ hour at 40°, produces a quantity of reducing matter which in regard to reducing capacity is equivalent to 120 mg. of glucose. The determination is actually carried out with 1 cc. of plasma (or serum), which is incubated with 5 cc. of starch paste and 2 cc. of a 1% NaCl solution for 30 minutes, and subsequently deproteinized by our copper method. The reduction value determined in the filtrate, minus the original sugar content of the plasma, represents the diastase value. From the normal average figure of 120 considerable deviations are found in either direction. The lowest value is, with very few exceptions, 80, the highest about 180. But, while individual variations spread over a considerable range, the blood diastase of one and the same individual shows a remarkably constant level; it is, moreover, largely independent of nutritional factors and does not change even over periods of months and years.

During the past 2½ years we have been running blood diastase determinations on nearly 100 healthy persons and several hundred hospital patients, in order to gather information in regard to possible deviations from the normal values. At this occasion we wish to deal only with conditions in which the diastase level is subnormal. As regards conditions which entail considerable augmentation of the blood diastase, fruitful studies have been conducted by previous workers. As to subnormal diastase values and their possible significance, however, few if any reliable observations were made, due, we believe, to the fact that the methods available would not only have failed to determine the low diastase values accurately, but also might have failed to permit the detection of these small values.

Early in the course of our work we found markedly low diastase values, ranging from 10 to 70, in icteric bloods and in the blood of pneumonia patients. Subsequently similar values were found in cases of toxemia of pregnancy, and regularly in connection with hepatitis, cirrhosis, abscess and carcinoma of the liver, as also with numerous cases of cholecystitis. Eventually we were led to the assumption that low blood diastase is found only in cases in which some form or other of liver damage, and consequent impairment of liver function, is present.

Clinical observations as well as histological examinations, performed after operations and autopsies, fully support our theory. In some cases the low level of blood diastase directed attention to liver pathology even before the existence of the pathological condition was revealed by the clinical picture. Again in other cases the fact that the blood diastase was normal was helpful in ruling out

liver damage. Successful medical or surgical treatment of pathologic liver conditions was in every instance promptly followed by a rise of the subnormal diastase level which soon attained a normal value.

7755 P

Influence of Certain Foodstuffs on Lesions of Endamoeba Histolytica Infection.*

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From the Department of Tropical Medicine, Tulane University, New Orleans, La.

It has been shown[1, 2] that raw liver and liver extract are distinctly beneficial to dogs suffering from acute amebic enteritis. On the other hand, ventriculin was found to be consistently harmful to the host. Furthermore, one of us (E.C.F.) discovered several years ago that dogs which were resistant to amebic infection on a balanced diet, could usually be infected when they were fed canned salmon. Our present inquiry is directed to the nature of the complex relations produced by these 3 foodstuffs on the wall of the large intestine, when the host has amebic enteritis. This report outlines our findings up to the present time and offers no explanations for the results obtained.

Twenty-six healthy young dogs have been used in the study. None were naturally infected with amebae. All were inoculated intracecally[3] with the same human strain of *Endamoeba histolytica*. All were suffering from acute amebiasis of a few days' standing when the tests were made. Fresh pig's liver, ventriculin (furnished by Parke, Davis and Co.) and commercial canned pink salmon (grade B) were the foodstuffs employed. Only one animal died; the remainder were sacrificed.

In the liver series 150 gm. of unchopped raw liver were fed to one animal daily. Clinical improvement began about the ninth day, and on sacrifice 13 days later only a few small shallow amebic lesions were found in the cecum and rectum. In contrast, when only 60 gm. of finely chopped liver in liver juice were fed to the

* Aided by a grant from the David Trautman Schwartz Research Fund.

1 Kagy, E. S., and Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 252.

2 Faust, E. C., and Kagy, E. S., *Am. J. Trop. Med.*, 1934, **14**, 235.

3 Faust, E. C., *Porto Rico J. Pub. Health and Trop. Med.*, 1931, **6**, 391.

host, improvement was noted on the fifth day, and on sacrifice 3 days later only a few shallow lesions were seen in the rectum. In 4 animals both the liquid and solid fractions of finely chopped liver were introduced into the large intestine. When only 12 gm. were used, 2 dogs showed clinical improvement beginning on the fourth day, and one failed to improve. Autopsy revealed only a few amebic lesions in the rectum of one host, numerous shallow lesions in the large intestine of the other 2. In a fourth dog, in which the liver, administered intracecally, was increased to 60 gm. *per diem*, marked improvement was noted on the third day and on sacrifice 2 days later very few pinpoint lesions were discovered. In another group finely chopped liver was autoclaved at 17 pounds pressure for 20 minutes and 60 gm. administered orally (2 animals) and intracecally (2 animals) *per diem*. All of these dogs became rapidly worse, were sacrificed on the eighth day, and were found to have multiple lesions throughout the large intestine, with many motile amebae in the lesions and in the lumen. In a third group the chopped liver was heated at 70°C. for 30 minutes to coagulate all proteins. The solid fraction, doubly filtered and washed, was fed orally in the amount of 85 gm. *per diem* to each of 2 dogs. The liquid fraction (250 cc. solution from 100 gm. raw liver) was also administered orally to each of 2 dogs. All 4 animals were sacrificed on the twenty-second day. Those receiving the solid fraction showed no improvement and at autopsy had numerous deep, undermining lesions, without healing. The other 2 had only a very few small, shallow amebic foci, with extensive healing.

In the ventriculin series in one group the commercial product was suspended in water and 10 gm. in 100 cc. of water were administered orally (one dog) and intracecally (2 dogs) *per diem*. In all 3 animals the infection became rapidly fulminating and on sacrifice (two on the 9th day and one on the 12th day), multiple deep amebic lesions were found throughout the large intestine of each dog and in one a general inflammatory condition was seen. In a second group the ventriculin, suspended in water and autoclaved (17 pounds pressure for 20 minutes), was then administered orally (2 animals) and intracecally (2 animals) in the amount of 6 gm. (suspended in 50 cc. of water) *per diem*. In all 4 of the animals improvement occurred and on sacrifice on the ninth day relatively few active lesions were found.

In the salmon series oral administration of the unaltered canned product was used routinely to exacerbate mild chronic or inactive infections. When, however, the unaltered product was macerated

and introduced intracecally, the host noticeably improved. On return to oral administration the infection promptly fulminated. When peptic and tryptic digests of salmon were introduced intracecally (30 cc. containing 25 gm. of the canned product *per diem*), the dogs became seriously ill on the sixth day and the condition rapidly fulminated. On sacrifice on the tenth day the large intestine of each dog was studded with amebic lesions.

In addition, liver and ventriculin (15 gm. each, suspended in 100 cc. of water *per diem*), and liver and salmon (15 gm. each *per diem*) were combined and administered intracecally. In the former experiment the liver failed to counteract the effect of the ventriculin but in the latter marked clinical improvement and recovery were effected and on sacrifice (on the 12th day) no amebae and no unhealed lesions were discovered.

7756 P

Water Balance in Adrenal Insufficiency and Inanition.*

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Recent reports of water balance studies during adrenal insufficiency in the dog[1, 2, 3] indicate that in that animal urine volumes, following cessation of cortin injections in adrenalectomized animals, are maintained at or above normal levels in spite of lowered water intake, with little or no reduction until shortly before death. Harrop, *et al.*,[2] attribute the hemoconcentration which occurs during adrenal insufficiency to loss of fluid by way of the kidneys, and Swingle, *et al.*,[3] also regard such loss as an important factor in reduction of plasma volume, though not the sole one.

The experiments herein reported were undertaken to ascertain whether similar results could be obtained with the cat, and whether

* Aided by a grant from the Carnegie Corporation of New York to the Carnegie Institution of Washington.

[1] Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., *J. Exp. Med.*, 1933, **57**, 775.

[2] Harrop, G. A., Weinstein, A., Soffer, L. J., and Trescher, J. H., *J. Exp. Med.*, 1933, **58**, 1.

[3] Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M., *Am. J. Physiol.*, 1934, **108**, 428.

any changes in water balance could be specifically referred to adrenal insufficiency.

Seven cats were used in these experiments; 3 of them were adrenalectomized and were on a maintenance dosage of cortin before experiments began; the other 4 had one adrenal out in the beginning, and the second adrenal was removed during the course of the investigation. One cat was followed not only during 2 such periods of adrenal insufficiency, but also during one period of recovery following cortin deficiency. Two cats were used during periods of gradual reduction of food intake simulating the reduction which occurs in the development of adrenal insufficiency, then a period of increasing food intake simulating recovery from insufficiency; finally the one remaining adrenal was removed and the same animals studied during the development of cortical insufficiency.

The water intake in food and drink was measured, and the metabolic water calculated. The water output in urine and feces was measured, and in addition a rough estimate of the insensible loss was made from the data on intake and output and the daily weight changes of the animal.

In contrast to the results reported by other investigators for dogs, the cats in these experiments invariably showed a decrease in urine volume immediately on the onset of adrenal insufficiency, the decrease accompanying the reduced intake. However, the reduction in intake was slightly more rapid than that in output, so that the water balance was relatively negative during cortical deficiency as compared with the normal period, a finding in agreement with Swingle, *et al.*[3]

During recovery from adrenal insufficiency, water intake increases somewhat more rapidly than does the output, so that the curves for the recovery period are the reciprocal of those for the development of insufficiency. This is in marked contrast with the results obtained on dogs by Swingle[3] who stated that the water balance was still more strongly negative during recovery than during insufficiency.

The water balance results obtained on cats during adrenal insufficiency and recovery were duplicated on animals in good health with only one adrenal removed, by gradually reducing food intake, and then increasing it again. This finding indicates that the water balance conditions of the total organism have no specific relation to adrenal insufficiency, but are merely a consequence of the nutritional state. Therefore, the hemoconcentration of adrenal insufficiency can not be even partially explained, in the cat at least, by loss of

fluid by way of the kidneys, but the explanation must come from a study of the factors involved in the abnormal distribution of water within the body. That the total water exchange can not account for all the symptoms of adrenal insufficiency is further indicated by the work of Caldwell,[4] who found that the water content of adult cats could be reduced practically to the vital limit without disturbance in the thermoregulatory mechanism, whereas such disturbance is one of the notable features of cortical deficiency.[5, 6]

The values found for insensible weight loss do not differ significantly during adrenal insufficiency from the corresponding values during the normal period. This is interesting in view of the lowered metabolism during cortical deficiency, and the view expressed by some authors[7] that insensible weight loss follows the basal metabolism so closely that the former can be used as a measure of the latter.

7757 C

Anti-Gonadotropic Substances.

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Both the gonadotropic hormone prepared from the pituitary gland and the anterior pituitary-like hormone of pregnancy urine (A.P.L.) lose their gonad-stimulating effect after a certain time, if given daily over a long period, but this loss of sensitivity is limited to the gonadotropic preparation with which the animals have been injected previously. Animals which become insensitive to pituitary implants remain sensitive to A.P.L. and *vice versa*.[1, 2] It has also been found that a state of passive A.P.L. resistance may be induced in immature female rats by the administration of the blood of A.P.L.-resistant

[4] Caldwell, G. T., *Physiol. Zool.*, 1931, **4**, 324.

[5] Hartman, F. A., Brownell, K. A., and Crosby, A. A., *Am. J. Physiol.*, 1931, **98**, 674.

[6] Hartman, F. A., Brownell, K. A., and Lockwood, J. E., *Endocrinology*, 1932, **16**, 521.

[7] Benedict, F. G., and Root, H. F., *Arch. Int. Med.*, 1926, **38**, 1.

[1] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

[2] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

animals[3] and these findings have recently been confirmed in the rabbit.[4] We here report our more recent experiments concerning the production of sera inhibitory to the gonadotropic hormone of the pituitary and to A.P.L.

Anti-A.P.L. The technique of obtaining and assaying the inhibitory sera followed closely the methods previously described.[3] Adult rats were injected daily with doses of A.P.L. increasing from 10 to 40 R.U. for periods of from 35 to 60 days. Their blood was collected 48 hours after the last injection, defibrinated, and the sera pooled. A typical assay consisted of 6 daily subcutaneous or intraperitoneal injections of 1 cc. of such sera into each of five 21-day-old female rats. During the last 3 days, A.P.L. in doses varying from 2 to 6 R.U. per day were also given. Control animals received only A.P.L. or pooled normal rat serum and A.P.L. The vaginal smears and the ovaries were examined microscopically.

It was found that the sera of 7 adult intact female rats having 50 days of A.P.L. treatment with a total individual dosage of 1400 R.U. completely inhibited vaginal oestrus and corpus luteum formation in immature females treated with 9 R.U. of A.P.L. The weight of the donors' ovaries averaged 48 mg. The sera of a parallel group of 7 similarly treated females was still inhibitory 70 days after treatment was stopped, the donors' ovaries averaging 39 mg. at the time.

In another group of female rats chronically treated with A.P.L. we found that loss of sensitivity to this hormone did not interfere with conception, but pregnancy tended to end in death and resorption of the foetuses during the last half of gestation. The sera of 2 such A.P.L. treated pregnant groups, one with living and one with dead ova, were assayed and found inhibitory.

Inhibitory substances were also demonstrated in the sera of a group of 8 adult castrate females after treatment for 44 days with a total of 750 R.U.-A.P.L., 10 normal adult males after 34 days and 1300 R.U.-A.P.L., and 10 adult castrate males after 32 days and 960 R.U.-A.P.L.

It is also possible to use the rabbit for the production of inhibitory sera.[4, 5] In most of our experiments with rabbits we have employed ascending (100 to 5000 R.U.) daily intravenous injections of A.P.L. in 2 courses of 5 injections each, separated by

[3] Selye, H., Bachman, C., Thomson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113.

[4] Twambly, G. H., and Ferguson, R. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 69.

[5] Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **32**, 28.

a rest period of 5 days. The material to date comprises 22 adult females treated by this short method, and 3 adult females, 1 adult castrate female and one adult male treated with small (1-100 R.U.) daily doses for 6 months.

Rabbits given large doses of A.P.L. by the above method showed very little continued gonadal stimulation after the 5th day. By the 10th day all corpora hemorrhagica and lutea present were apparently ageing. Blood drawn within 48 hours of the last treatment, and injected in 1 cc. doses for 4 days into immature female rats provoked oestrus and corpus luteum formation. Blood drawn from the 4th to the 10th day after treatment has proven uniformly and strongly inhibitory in 9 assays to date on the immature rat, using test dosages of A.P.L. up to 60 R.U. Blood drawn one and 2 months after treatment has been found to be no longer inhibitory. The inhibitory titer of such donor rabbits has again been raised by a course of 5 daily A.P.L. injections.

The normal female rabbit has been found to be a suitable test object for anti-A.P.L. substances. Four adolescent virgin does of over 1800 gm. body weight, and 4 non-pregnant isolated parous females were given A.P.L. in 4 intravenous doses of 25 R.U. at intervals of 12 hours, each such dose preceded 2 hours earlier by the injection of 1 cc. of anti-A.P.L. rabbit serum into the opposite ear vein. Twenty-four hours after the last injection their ovaries showed no change compared with the condition in which we found them at a preliminary laparatomy before the experiment was begun. Four control animals given only the A.P.L. all showed numerous large corpora hemorrhagica and a few apical corpora lutea.

Anti-Maturity. The assays of sera for substances inhibitory to gonadotropic extracts of the anterior pituitary have been modelled on those described above. A standard dosage of 0.25 cc. of an alkaline extract of sheep pituitary, 1 cc. representing 0.25 gm. of anterior lobe given subcutaneously twice daily for 3 days, has been used. Such a dosage has given ovaries averaging 40 mg. in the control animals.

Serum donor rats were treated for 6 to 10 weeks with twice daily subcutaneous doses of 0.25 to 1.0 cc. of the same and similar extracts prepared from hog pituitaries.

Under the conditions of assay it was found that the anti-maturity potency of donor sera from treated adult female rats was independent of the length of treatment, but appeared to parallel the development of the refractory state as evidenced by eventual regression in the weight of the donors' ovaries.

A group of 18 animals treated for 10 weeks was divisible into 3 groups: One group of 8 rats had ovaries averaging 26 mg.; the pooled sera of this group inhibited the action of maturity hormone in the immature female assay animals. In the second group of 7, and the third group of 3 animals, with average ovarian weights of 66 and 267 mg. respectively, the sera failed to show inhibitory potency.

Summary. From these experiments we conclude that the chronic administration of gonadotropic extracts from the pituitary or from pregnancy urine leads to the formation of substances inhibitory to their action, and that a passive resistance to both these hormones may be produced by the administration of serum obtained from animals chronically injected with these gonadotropic substances.

7758 P

Total Creatinine Content of Perfused Rabbit Hearts.

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In our efforts to determine the part played by creatine and its possible precursors in heart muscle metabolism, we perfused isolated rabbit hearts in Dawson's modification[1] of the Gunn-Locke apparatus. We used oxygenated Ringer-Locke solution to which 0.1% dextrose was added in one apparatus, and in the other the same solution plus 0.1% glycocoll. About 250 cc. of each mixture was placed in the reservoirs, and that which perfused through the coronaries was collected and returned automatically to the reservoirs and oxygenated by an oxygen pump system.

Thirty isolated rabbit hearts, or 15 pairs, were perfused from 1 to 5 hours, beating spontaneously, or stimulated at the rate of 60 per minute when spontaneous contraction was too slow. Thirteen isolated hearts were perfused for a minute each in order to wash the blood from the coronary system, and used as controls. All hearts were weighed, cleaned of fat and connective tissue, and the ventricular muscle minced. Part of the tissue was dried at 105°C. for 22 hours to give the percent of solids; the remainder was used for determination of the total creatinine content by the method of

[1] Dawson, W. T., *J. Lab. and Clin. Med.*, 1925, **10**, 853.

Rose, Helmer and Chanutin.[2] Because of the variable amount of edema produced during the perfusion, it was thought desirable to calculate the results in terms of the dry weight.

In the control hearts, the total creatinine content varied from 144 to 168, with an average of 153 mg. per 100 gm. of muscle. In terms of dry weight, there were from 753 to 868, with an average of 795 mg. per 100 gm. of dried muscle.

In the hearts perfused with Ringer-Locke solution alone, the total creatinine values ranged from 100 to 151, with an average of 123 mg. per 100 gm. of moist muscle; or, from 652 to 827, with an average of 742 mg. per 100 gm. of dried muscle. In the series perfused with Ringer-Locke to which glycocoll had been added, the values ranged from 103 to 159, with an average of 126 mg. per 100 gm. of moist muscle; or, from 623 to 855, with an average of 739 mg. per 100 gm. of dried muscle.

Under our experimental conditions, the addition of glycocoll to the perfusion fluid had no evident influence on the total creatinine metabolism of the heart muscle. The fact which we observed, that in most instances the hearts perfused with a fluid containing glycocoll beat more vigorously, and maintained a spontaneous rhythm for a longer period than did those perfused with Ringer-Locke alone, may be explained by assuming a specific stimulating action of the amino-acid. This has been suggested to us by Prof. B. M. Hendrix as the possible explanation of the effect of glycocoll in diseases of striated muscle.

We are indebted to Prof. W. T. Dawson for the loan of the apparatus.

7759 P

Comparison of a Rapid (Folger-Solé) Method and the Routine Loeffler's Method for Diagnosis of Diphtheria.

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Recently a paper by Solé[1] describing his diagnostic results in 200 cases of diphtheria, by a rapid cultural method originally suggested by Folger some 35 years ago but never published, inspired us to

[2] Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

[1] Solé, Alphons, *Wien. Klin. Wchschft.*, 1934, **47**, 713.

apply the procedure with certain modifications to the problem as it presented itself to us at the Willard Parker Hospital. For many years we have been confronted by the difficulty of getting a sufficiently prompt and definite bacteriologic diagnosis in clinical diphtheria, or in ruling out diphtheria in carrier suspects within a reasonable length of time, by the classical Loeffler's blood serum slant method. By contrast, with the herein described rapid method we have been able to secure 95% positive cultures by the end of 4 hours.

The technique employed is extremely simple. Sterile cotton swabs are impregnated thoroughly with undiluted, unheated, horse-serum to which no preservative has been added. The swabs are then squeezed lightly against the sides of the tube to remove any surplus serum. They are removed and lightly heated over a flame to secure at least surface coagulation, and possibly as Solé says to destroy any serum antibodies. These swabs are then utilized to take the ordinary routine nose and throat cultures of the suspected case. They are placed in dry sterile tubes in the incubator and examined at the end of 2 and 4 hours by smear preparations. The slides are stained routinely by Ponder's method. They are graded as negative, suspicious or positive. In the tabulation presented here only undoubted positives are included. Too much emphasis cannot be placed upon the care with which these cultures should be taken, in order to secure satisfactory specimens. At the end of the 4-hour period, Loeffler's slants were routinely inoculated from these swabs, for isolation, control and subsequent identification by fermentation.

For convenience in interpreting our results the cases were divided into 3 groups: (1) cases of clinical diphtheria, (2) diphtheria carriers, and (3) negative controls (including streptococcic laryngitis, observation scarlatina, etc.) In respect to the first of these groups —the cases of clinical diphtheria—the results have been incorporated in Table I.

Of the 72 cultures representing 38 cases of diphtheria, 66 were positive. As may be seen from the chart, 34 or 92% of the throat cultures were positive at the end of 4 hours as compared with 28 or 76% by the Loeffler method at the end of 18 hours. Of the nose cultures only one represented a simple nasal diphtheria which accounts in part for the lower figures. The 38th case of this group was a frank clinical case of toxic diphtheria with membrane in which neither the hospital laboratory nor the Department of Health diagnostic laboratories were able to secure positive cultures. The second part of the table analyzes these figures in more detail.

In respect to the second or carrier group. We had 13 such cases

TABLE I.

Clinical Diphtheria—38 Cases. Comparative Results—Routine and Rapid Methods.

	No. of cultures	No. of positive KL cultures	Rapid Method Positive in 2 hrs. No.	%	4 hrs. No.	%	18 hrs. No.	%	Loeffler Slant Routine Method Positive in 18 hrs. No.	%	Comparison of Results: Loeffler's 18-Hour Slant Method vs. Rapid Method: Negative Loeffler Positive Rapid 18 hrs. No.	%	Negative Loeffler Positive Rapid 4 hrs. No.	%	Positive Loeffler Negative Rapid 4 hrs. No.	%	Positive Loeffler Positive Rapid 4 hrs. No.	%	Negative Loeffler Negative Rapid No.	%
Nose	34	29	13	45	17	59	27	93	11	38	18	53	8	24	2	6	9	27	5†	15
Throat	38	37	29	78	34	92	37	100	28	76	9	24	6	16	0	0	28	74	1*	3
Total	72	66	42	64	51	77	64	95	39	59	27	38	14	19	2	3	37	51	6	8
Total cases	38	37	30	81	35	95	37	100	31	84	6	16	4	11	0	0	31	82	1*	3

*Case had uniformly negative cultures, but clinically was considered toxic diphtheria.

†Cases of tonsillar or pharyngeal diphtheria with negative nasal cultures.

with 34 separate cultures in this series. Of the 34 cultures 29 were considered positive in the final analysis. Of these, 24 or 83% were isolated by the 4-hour method, whereas only 19 or 65% were identified as positive by the usual Loeffler's slant method at the end of 18 hours.

The particular point of interest in this group is the further fact that by this rapid method with a subplant to Loeffler's from the swab at the end of the 4-hour period, we can secure within 18 hours a practically pure culture suitable for the toxicity test.

In the third or control group there were 8 cases with 14 separate nose and throat cultures. These were uniformly negative both by the rapid method and the Loeffler method in our own hands and in the control reports from the Department of Health Laboratories, with the exception of a single nose culture reported positive by the Department of Health, but subsequently shown to be *B. hoffmanni* by fermentation reactions. This group needs no further comment.

Summary and Conclusions. A modified rapid cultural method for the diagnosis of clinical diphtheria is described whereby accurate reports may be rendered in over 80% of cases within 2 hours, and in 95% within 4 hours, as compared to the average of 83% accuracy at the end of 18 hours by the usual Loeffler's slant method. The method seems to us to be of great value in establishing early diagnosis and treatment in the disease. It further provides for the securing of pure cultures within 18 hours for toxicity tests, thus reducing the necessary period of isolation and possible exposure to true diphtheria for non-toxic carriers, a matter of grave importance, to patient and hospital alike. We feel that the method should supplant the older, more cumbersome procedure in all contagious hospitals, and that it should be utilized very largely by health departments both in their diagnostic and release cultures as an adjunct to their present methods. We are convinced that by earlier diagnosis and treatment the mortality of the disease should be further reduced. We believe that the carrier problem is materially improved with appreciable effective saving of hospital days to both the individual and the institution.

7760 P

Characteristics of Mucoid-Encapsulated Organisms Isolated from Cases of Bronchial Asthma.

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Sixty-nine cultures from cases of bronchial asthma were studied and 28 found to be positive for mucoid encapsulated bacilli. There were 9 positives from the nose, 14 from the throat, 4 from sputa and 1 from blood. Cultures were made from swabbings from the upper respiratory tract and in one instance from the blood. Swabbings were first cultured directly on blood agar plates. The swabs were then put into tubes of Avery's medium and incubated over night. If the gram stain for organisms grown in Avery's medium showed the presence of gram negative encapsulated bacilli the following day cultures were made from the Avery's tubes to blood agar plates. With 2 exceptions there was poor growth of the mucoid encapsulated organisms cultured directly from the swabbings to blood agar plates. The organisms grew well in Avery's medium and later when transferred grew well on the blood agar plates.

The organisms described in these studies were gram negative, encapsulated, non-sporulating bacilli. There was a great variation in size and shape, ranging from coccoid to long rod forms in the same cultures. On *agar* the colonies were large and grayish white in color. Viscosity varied with the individual strains. In *broth* there was a general cloudiness of the medium with a heavy stringy sediment. In *gelatin* there was a grayish white mucoid growth on the surface. With the exception of one case there was no liquefaction of the gelatin.

Fermentation reactions were variable and in accordance with similar reactions studied by other investigators (Small and Julianelle,[1] Fitzgerald,[2] Clairmont,[3] Perkins[4]). Dextrose, mannite, lactose, saccharose, maltose, galactose, raffinose, rhamnose, sorbitol, salicin, inulin, dulcitol and inositol were used. The majority of the organisms isolated fermented dextrose, mannite, lactose, saccharose, maltose, sorbitol and salicin. With these carbohydrates there was

[1] Small, J. C., and Julianelle, L. A., *J. Infect. Dis.*, 1923, **32**, 456.

[2] Fitzgerald, J. G., *J. Infect. Dis.*, 1914, **15**, 268.

[3] Clairmont, P., *Z. Hyg. Infekt. Kr.*, 1902, **39**, 1.

[4] Perkins, R. G., *J. Exp. Med.*, 1900, **5**, 389; *J. Infect. Dis.*, 1904, **1**, 241.

gas formation as well as fermentation with acid. Galactose, raffinose and rhamnose were fermented with acid formation but gas production was quite variable. Inulin was fermented in only one instance.

In *milk* acid was formed in most instances though with 2 strains an alkaline reaction occurred. Coagulation took place in a few instances. Indole production was absent in all but 3 cases.

In general it will be noted that the organism studied in these cases of bronchial asthma gave typical cultural characteristics, morphology and biochemical reactions of members of the mucoid encapsulated group of bacilli.[5] When present in bronchial asthma there was an associated hypersensitivity of the patients towards the intra-dermal test with vaccine prepared from the bacilli.

7761 C

Augmentation of the Positive After-Potential of Nerves by Yohimbine.

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A nerve poisoned by yohimbine exhibits, after a single response, a recovery curve of excitability characterized by a refractory period, a supernormal period, and a subnormal period.[1] Of these the first 2 have been recognized as existing in unpoisoned nerve and have been brought into approximate relationship with the parts of the action-potential known as the spike and negative after-potential. A subnormal period has been recognized in unpoisoned nerve but only after the nerve has been tetanized, in which case the subnormality is associated with the positive after-potential.[2] The latter association suggests that the effect of yohimbine is to augment the process responsible for the positive after-potential. Such being the case, the apparent absence of a subnormal period following a single response in unpoisoned nerve would be interpreted as due to the small size of the positive after-potential which there exists.

[5] The Mucoid-Encapsulated Group, A System of Bact. in Relation to Medicine, Med. Research Council, His Majesty's Stationery Office, London, 1929, **4**, 289.

[1] Graham, H. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 193.

[2] Gasser, H. S., *Am. J. Physiol.*, 1934, in press.

A few experiments sufficed to demonstrate that the potential is augmented as was anticipated. The nerves (isolated sciatic of *Rana pipiens*) were treated in all cases as in the excitability experiments, and their potentials recorded on a cathode ray oscillograph after amplification with a direct-current amplifier, the latter being necessary to avoid distortion of potentials of the length in question.

The progress of the potential-change can best be followed in connection with Fig. 1. All parts of the figure start from a potential-

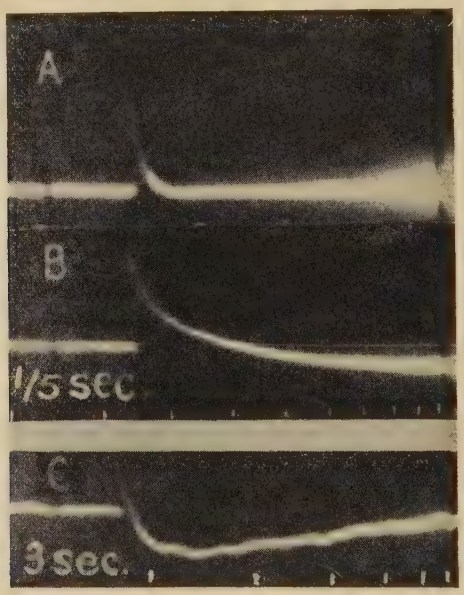

Fig. 1. Effect of yohimbine on after-potentials: A and B on the same time scale and at same amplification; A, normal; B, after yohimbine; C, same condition as B recorded with slower sweep and greater amplification. The nerve is killed under the distal lead. An upward deflection in all records means that the proximal (active) lead is negative. Temperature 24°C.

level reached after a long period (15 minutes or more) of freedom from activity. Activity is induced by a single break induction shock and starts with the spike potential, which throws the spot far off the record. The actual record starts with the negative after-potential and is continued by the positive. In the initial unpoisoned state (Fig. 1 A) the positive after-potential reaches a maximum of about 5μv and is not distinguishable for more than about 0.6 sec. In the period after yohimbinization it becomes larger and longer (Fig. 1 B). As shown in Fig. 1 C, it reaches a maximum potential of 33μv at 1.7 sec. and has only dropped to one-third of the maximum after 15 sec. Other experiments resemble the one cited, though

there is a considerable variation. Maximum positivity usually occurs in the neighborhood of one second. Half restoration comes commonly in the range 6 to 9 sec., and the total duration may be one-half minute or more. There is thus good correlation with the dimensions of the subnormal period manifested by yohimbinized nerve.[2] The curves reproduced in Fig. 1 show an increase in the duration of the negative after-potential. This is a spontaneous increase which occurs characteristically in all isolated nerves. It is not typical of yohimbine action. When the negative after-potential has developed *before* the application of the drug the effect of the latter is to decrease and shorten it.[1]

As holds for the positive after-potential of normal nerve, accumulation of potential occurs during and after tetanization of yohimbinized nerve. Compared with normal nerve there is a striking difference, however. In the latter the negative after-potential keeps pace with the positive so that during the tetanus the level of the negative after-potential is maintained approximately constant at its maximum. In yohimbinized nerve, on the contrary, the positive potential is so dominant over the negative that, in spite of a constant contribution of negative after-potential by each succeeding impulse in the tetanus, the algebraic sum of the 2 potentials becomes increasingly positive and the

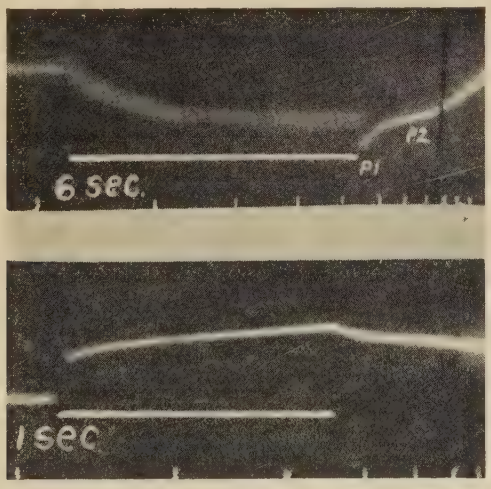

FIG. 2. (Top.) Positive after-potential during and after a tetanus of a yohimbinized nerve. Period of the tetanus marked by white line. Frequency of stimulation about 5 per sec. 1 cm. deflection = 0.52 *mv*. Temperature 22°C.

FIG. 3. (Bottom.) After-potential during and after a tetanus of a veratrinized nerve. Frequency of stimulation 20 per sec. Nerve at room temperature, about 23°C.

negative after-potentials fail to pass the zero level even at their maxima. The course of events is illustrated in Fig. 2. At the break in the line the tetanus starts. Only the after-potentials are visible, the width of the white band of the record being determined by the increments of negative after-potential. If swept out the component responses would look like Fig. 1 B. The top of the band is determined by the negative after-potential crest and the bottom of the band by the amount to which the potential has subsided at the time of arrival of the succeeding impulse. As the tetanus proceeds the positivity at first increases; then the 2 potentials come into balance and a steady state is maintained on the positive side of zero. When the tetanus ceases the negative after-potential subsides much earlier, revealing the positive potential unopposed. In the subsequent restoration of the potential to the resting value 2 parts are seen which are also characteristic of unpoisoned nerve[2]: a rapid portion manifest initially *(P1)* and a slower portion which completes the restoration *(P2)*.

For contrast with the potential accumulation in a yohimbinized nerve during a tetanus, the accumulation of potential as seen in a veratrinized nerve in a similar experiment is shown in Fig. 3. Negative after-potential is here dominant, and the level of the latter rises continuously during the tetanus and lasts long after the latter has ended. In fact, in some nerves the crest of the negative after-potential is not attained until after the tetanus is over.

7762 C

A Fermentation Inhibiting Substance Produced by B. Coli.

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When diphtheria bacilli were grown in broth media in which colon bacilli had previously been cultivated, the fermentation of dextrose, galactose and dextrin was inhibited or retarded. Usually this retardation was evident over a period of about 7 days and at the end of this time fermentation began to become evident. This inhibitory effect of the previous growth of *B. coli* on the fermentative activity of *C. diphtheria* was only evident when small batches of media (150 to 175 cc.) were exposed to the action of *B. coli*. In larger batches of media this inhibitory effect did not develop.

Colon bacilli were inoculated into sterile solutions of peptone, beef extract or meat infusion (both before and after removing solids and coagulable material) plus 0.5% sodium chloride and then incubated 48 hours. The media were centrifuged to remove bacteria as far as possible; the ingredient lacking (either peptone or beef extract) was added, the reaction brought to pH 7.4-7.6 and bromcresol-purple was added as indicator. The media were then filtered through either Berkefeld or Seitz filters to render them sterile. The carbohydrates were added either before or after filtration in quantities sufficient to make a 1% concentration.

The inhibiting effect on fermentation by diphtheria bacilli was evident in all media prepared as above; the results of one experiment are shown in Table I. Six different strains of *B. coli* of the fecal

TABLE I.

	Diphtheria Culture No.	Days of Incubation. Dextrose 1	2	4	7	Galactose 1	2	4	7	Dextrin 1	2	4	7
B. coli	1	—	—	—	A—	—	—	—	—	—	—	—	—
filtrate	3	—	—	—	—	—	—	—	—	—	—	—	—
in nutrient	12	—	—	—	A—	—	—	—	—	—	—	—	—
broth	19	—	—	±	A	—	—	—	—	—	—	±	A
B. coli	1	—	—	—	A	—	—	—	±	—	—	—	A
filtrate in	3	—	—	—	—	—	—	—	—	—	—	—	—
peptone	12	±	A	A	A	—	—	±	A	—	—	±	A
water	19	—	—	—	A	—	—	—	±	—	—	—	—
B. coli	1	—	—	—	A	—	—	—	—	—	—	±	A
filtrate	3	—	—	±	A	—	—	±	A	—	—	—	—
in beef	12	—	—	±	A	—	—	—	±	—	—	A	A
extract	19	—	—	±	A	—	—	A	A	—	—	—	A
Control	1	A	A	A	A	—	A	A	A	—	A—	A	A
plain	3	A	A	A	A	±	A	A	A	±	A	A	A
broth	12	A	A	A	A	A	A	A	A	A	A	A	A
	19	A	A	A	A	A	A	A	A	A	A	A	A

— No acid produced. ± Slightly acid. A Acid.

type were used and all produced essentially the same inhibiting effect. Several strains of *C. diphtheriae* were tested and the influence of the filtrate was evident on the fermentation activity of all. There were slight variations in the production of inhibiting influence by the strains of colon bacilli and also in the resistance of the individual strains of diphtheria bacilli to the inhibition. These colon bacilli filtrates did not interfere with the multiplication of the diphtheria bacilli.

An experiment was performed to determine whether the *B. coli* had exhausted the peptone. Peptone in solid form and in sufficient

quantity to make a 0.5% solution was added to a broth filtrate or peptone water filtrate in which *B. coli* had been grown. In this media the inhibiting effect was also evident.

Since many of the proteolytic enzymes of *B. coli* are extracellular, the filtrate of a 30-hour culture of *B. coli* was added to sterile broth and peptone water and incubated for 3 days. The mixture became slightly alkaline, it was adjusted to pH 7.4-7.6; lacking ingredients were added as well as the indicator and the carbohydrates. When such media were inoculated with *C. diphtheriae,* fermentation of the three sugars proceeded as in the control tubes.

In order to determine whether the diphtheria bacilli were altered by growth in the *B. coli* filtrate, cultures of *C. diphtheriae* were made on blood agar plates from tubes in which fermentation had been inhibited; the colonies on these plates were identical with the original colony forms, and when these organisms were inoculated into control carbohydrate media the fermentation was as active as in the original culture. If, however, the diphtheria organisms were inoculated directly from filtrate media, in which inhibition had occurred, into control carbohydrate media, there was evidence of a slight retardation of the fermentative activity of *C. diphtheriae,* so that in some cases 48 to 72 hours were required for activity to appear; if a second transfer was now made to normal carbohydrate media, fermentation of the 3 carbohydrates occurred within 24 hours. If the *C. diphtheriae* were transferred into normal control carbohydrate media from the *B. coli* filtrate media after 7 days' incubation, little or no inhibiting effect was evident. It is probable that the inhibiting effect had been destroyed or neutralized at this time, since this corresponds to the time at which fermentation of the carbohydrates begins in the *B. coli* filtrate media.

Heating the *B. coli* filtrate showed that the inhibiting effect is not readily destroyed by heat. It is apparent that the variation in influence of the inhibiting action on different strains of diphtheria bacilli plays a rôle. In addition, heat apparently destroys most readily the inhibitory action in relation to dextrose, and least readily the action in relation to dextrin. Even when heated to 115-120°C. the inhibiting action of the *B. coli* filtrate may not be completely destroyed.

It was also found that when the filtrate was diluted with normal nutrient broth it was necessary to have present more than 50% of the filtrate in order to have the inhibiting effect evident; so that usually when the filtrate constituted 75% of the mixture the effect was evident.

B. coli was grown in synthetic media (Mavor's and Uschinsky's); the filtrates so obtained from the synthetic media were each mixed with one part of nutrient broth, to aid growth. In both of these mixtures the fermentation by *C. diphtheriae* was not interfered with or retarded.

The *B. coli* filtrate was placed in contact with kaolin at various hydrogen-ion concentrations, but the inhibiting substance was not removed.

Summary. *B. coli* produces, when grown in small batches of nutrient broth, a relatively thermostable substance which inhibits or retards fermentation of dextrose, galactose, and dextrin by *C. diphtheriae*. The diphtheria bacilli seem to absorb this substance and also to destroy it, and there is no permanent effect of this substance upon the diphtheria bacilli. The action is not dependent upon exhaustion of nutrient substances or alteration in the pH of the media.

7763 P

Lead IV of the Electrocardiogram in Rheumatic Fever.

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The most important clinical problem which presents itself in patients suffering from rheumatic fever is the recognition of active cardiac involvement. Bedside observation is sometimes adequate for decisive diagnosis. Serial electrocardiography, with employment of the 3 standard leads, has proved to be a valuable method for following the effect of the rheumatic process on the heart.[1] We have found that the additional use of lead IV has, in certain instances, revealed evidence of active carditis when in the first 3 derivations either no form changes were apparent in successive records, or the alterations noted were regarded as equivocal.

Thirty-eight patients with acute rheumatic fever were studied in the wards of the Presbyterian Hospital. None of these patients were taking digitalis or quinidine, although to a majority salicylate or pyramidon was being given. Two hundred and ten electrocardiograms were taken. In recording lead IV, the right arm elec-

[1] Cohn, A. E., and Swift, H. F., *J. Exp. Med.*, 1924, **39**, 1.

trode was placed over the lower precordial region just to the left of the sternum, the left arm electrode being placed at a corresponding level in the back, to the left of the spine.[2] Form changes in lead IV which were considered to be abnormal were an upright T wave, a negative T wave deeper than 9 mm., a monophasic or notched QRS group, an R-T or S-T segment above the isoelectric line or one that was more than 2 mm. below the isoelectric level.[3] Changes in form occurring in records taken on the same patient at different times were regarded as indicating alterations in the state of the myocardium. By far the most frequently observed changes in lead IV were alterations in the direction or voltage of the T wave. In this series, no example of elevation of the R-T or S-T segment occurred. The signs described, while they show that the heart is affected, are not specific for rheumatic fever.

In 7 cases a change was noted in lead IV only. In 16 cases changes were observed in all 4 leads; but in a number of these records minor variations in the first 3 leads, especially slight changes in the T wave in lead III, were rendered significant by the finding of gross alterations in lead IV. In 5 cases changes were observed only in the 3 standard leads, whereas lead IV was not affected. In 10 cases no changes were seen in any lead.

The series is small and no significance is attached to the relative number of cases in which each of these occurrences was noted. It seems likely that had electrocardiograms been taken at more frequent intervals, the incidence of changes indicative of cardiac involvement would have been higher.

A single record only was taken on 34 ambulatory patients with rheumatic heart disease who came to the Vanderbilt Clinic. In 8, deviations from the normal were observed in lead IV only.[4] In 7, changes were seen in all 4 leads. In 11, changes were observed in the 3 standard leads only. In 18, no changes were recorded in any lead.

Seven additional cases observed in the hospital came to the autopsy table. In none of these were changes observed in lead IV only. In 4, changes were noted in the first 3 leads and the T wave in lead IV was upright. In 3 of these cases numerous Aschoff bodies were found in the heart muscle. In the fourth patient acute fibrinous pericarditis established the presence of active rheumatic fever,

[2] Wolferth, C. C., and Wood, F. C., *Am. J. Med. Sci.*, 1932, **183**, 30.

[3] Katz, L. N., and Kissin, M., *Am. Heart J.*, 1933, **8**, 595. Master, A. H., *Ibid.*, 1934, **9**, 511.

[4] Katz and Kissin (ref. 3) and others have made similar observations in single records.

although no Aschoff bodies were observed in the sections of the myocardium. In one case the 3 standard leads showed changes, whereas lead IV was normal. In this patient acute rheumatic pancarditis was present. In 2 cases in which active rheumatic heart disease was suspected during life, no changes were present in any of the 4 leads of the electrocardiogram. In neither instance were Aschoff bodies found at autopsy. One proved to be an example of verrucous endarteritis associated with quiescent valvular lesions. The second case was one of gonococcal endocarditis superimposed upon old, but inactive, rheumatic heart disease.

Summary. In acute rheumatic fever, lead IV of the electrocardiogram sometimes furnishes evidence of active carditis, when changes indicating myocardial involvement are not observed in the standard 3 leads. Frequently definite changes in lead IV render significant minor alterations in the first 3 leads which might otherwise be regarded as of doubtful importance. On occasion, changes denoting rheumatic lesions in the heart muscle may appear in the first 3 leads without change being present in lead IV. In ambulatory patients with rheumatic heart disease a single electrocardiogram may reveal evidence of myocardial damage in lead IV only. Obviously, a single record does not establish the presence of rheumatic activity in the heart.

Changes in the electrocardiogram characteristic of myocardial involvement were found in 5 patients whose hearts, at autopsy, showed the lesions of active rheumatism. In 2 cases in which active rheumatic carditis was suspected during life, but was not found at autopsy, the electrocardiograms were normal.

On the basis of these observations it is concluded that the use of lead IV of the electrocardiogram is of definite clinical value, as a supplement to the 3 standard leads, in the recognition of active myocardial involvement in rheumatic fever and in following its course.

7764 P

Effect of Atropine on Impaired Auriculoventricular Conduction in Rheumatic Fever.

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That auriculoventricular conduction is frequently impaired in rheumatic fever is now well known. It is generally assumed that this disturbance is due to the presence of rheumatic lesions in the region of the auriculoventricular node or bundle.

Atropine sulphate, in amounts varying from 1.5 to 3.0 mg., was injected intravenously in 12 patients suffering from acute rheumatic fever. In each instance the electrocardiogram showed impairment of A-V conduction which varied in degree from lengthening of the P-R interval above 0.20 second to complete heart block. None of these patients had received digitalis or quinidine. A control electrocardiogram was taken, and further records were made at intervals of 1 to 4 minutes during the half hour following the injection. The maximum effect usually occurred during the first 15 minutes. The action of the drug always disappeared completely in the course of 24 hours, when the conduction time resumed its former level or occasionally one slightly above it. No undue subjective discomfort was induced, although dryness of the mouth was usual; pupillary dilatation and flushing of the skin sometimes were noted.

Eighteen observations were made on the 12 rheumatic subjects and 6 observations were taken on 3 individuals who showed no evidence of heart disease.

Rheumatic Group. In 9 of the 12 patients, shortening of the conduction time was noted after atropine injection. In one case of incomplete block, dropped beats disappeared and the P-R interval was 0.23 second in each cycle. An example is given in Table I. The

TABLE I

Female, aged 38. Fever, polyarthritis, mitral stenosis, and insufficiency. Leucocytes, 11,000. Sedimentation rate of erythrocytes, 130.

Time	P-R Interval	Heart Rate
10:43 A. M.	0.24	90
10:47	*2 mg. atropine, intravenously*	
10:48	0.19	100
10:50	0.18	105
10:52	0.19	100
10:57	0.20	98
11:02	0.20	98
3:00 P. M.	0.21	85

increase in cardiac rate varied from 5 to 50 beats per minute. The decrease in conduction time ranged from 0.03 to 0.08 second. There was no direct relationship between the degree of acceleration of rate and the extent of shortening of conduction. In one case, an increase in heart rate of 5 beats per minute was associated with a decrease of 0.06 second in conduction time. In another instance, an increase of 50 beats per minute was accompanied by a decrease of 0.04 second in conduction time.

Approximately half of these patients were receiving aspirin at the time the observations were made. In a number of cases atropine was injected first when no aspirin was being given and then again after its administration, in amounts up to 7.5 gm. daily. The decrease in the duration of conduction was of the same order of magnitude whether aspirin was being given or not. The increase in cardiac rate following the injection of atropine tended to be less when aspirin was being given than when it was omitted.

In 3 patients the degree of block was unaffected by atropine. Two of these were instances of complete heart block. Atropine produced a marked acceleration in auricular rate in both, but complete A-V dissociation persisted. In one of these patients complete block has been present constantly for 4 months since the atropine study was made. In the third case, prolongation of the P-R interval was not affected by atropine. It seems likely that in these cases there was permanent fibrosis of the junctional tissues as the result of rheumatic lesions.

Three cases, in addition to impairment of A-V conduction, showed a delay in intraventricular conduction time. Atropine had no effect on this disturbance.

Normal Group. In 6 patients without heart disease, injection of atropine was followed by an acceleration in rate varying from 20 to 50 beats per minute. The decrease in conduction time ranged from 0.01 to 0.03 second.

Summary. Impairment of A-V conduction in rheumatic fever often, though not invariably, can be diminished or abolished by atropine. Inasmuch as acceleration in rate and decrease in conduction time do not always parallel one another in degree or duration, it appears that these 2 effects are not necessarily directly related. The exact mechanism involved is a matter for speculation. It is conceivable either that the release of the vagus lowers the threshhold for conduction through the junctional tissues, possibly through some chemical action; or that the strength of the excitatory impulse is augmented to such an extent that it can pass an area which offers

increased resistance. Further observations along these lines are in progress.

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Diastase in Milk.

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Béchamp in 1883 was the first to recognize the presence of diastase in human milk; at the same time in cow's milk he found no trace of this enzyme.[1] Bouchut[2] and Moro[3–5] confirmed the findings of Béchamp.

During the last decade a number of investigators have attempted to establish a quantitative diastase test as a means of detecting whether or not a milk had been pasteurized. Namely, diastase would be entirely or partly inactivated during pasteurization, the extent of its destruction depending on the temperature and the duration of heating. The methods used by these workers, while claiming to yield quantitative results, are quite crude in comparison to the qualitative methods of Béchamp and Bouchut. The more recent workers find diastase in the milk of practically all the mammals[6] examined and are able to determine diastatic activity in the presence of lead[7–9] and even mercury salts.[10] The latter fact is characteristic of the unreliability of these methods.

We approached the problem with analytical procedures, which in the instance of blood and urine proved to be adequate for the determination of very low as well as of high diastase values.

The method is in brief as follows: a 1.5% starch paste is prepared of pure commercial corn- or rice-starch (but not of soluble starch); 10 cc. of this starch paste and 4 cc. of a 1% NaCl solution

1 Béchamp, A., *Compt. rend.*, 1883, **96**, 1508.

2 Bouchut, E., *Hygiène de la première enfance*, Paris, 1885, 102. (Quoted in Moro's work.)

3 Moro, E., *Jahrb. f. Kinderheilkunde*, 1898, **47**, 342.

4 Moro, E., *Jahrb. f. Kinderheilkunde*, 1900, **52**, 524.

5 Moro, E., *Jahrb. f. Kinderheilkunde*, 1902, **56**, 391.

6 Chrzaszcz, T., and Goralowna, C., *Biochem. Z.*, 1925, **166**, 172.

7 Rothenfusser, S., *Z. Untersuch. Lebensm.*, 1930, **60**, 94.

8 Gould, B. S., *J. Dairy Sci.*, 1932, **15**, 230.

9 Weinstein, P., *Z. Untersuch. Lebensm.*, 1934, **68**, 73.

10 Kluge, H., *Z. Untersuch. Lebensm.*, 1933, **65**, 71.

are measured into a test tube, the mixture is warmed to 40°, and then 2 cc. of diluted milk are added. The extent of the dilution (usually 1:10 to 1:40) depends on a preliminary test, which is based upon the amyloclastic activity of the milk. The test tube is stoppered, its contents mixed, and incubated in a water bath at 40°. After 30 minutes' incubation the proteins are precipitated by Somogyi's copper or zinc method,[11, 12] and the reducing matter in the filtrate determined by the Shaffer-Hartmann-Somogyi method.[13] From the reduction value thus obtained the original lactose content of the milk is subtracted.

Our results confirm, at least from the qualitative point of view, the observations of Béchamp, *i. e.,* human milk contains considerable quantities of diastase, while in cow's milk this enzyme is entirely absent. We have examined cow's milk in all stages of lactation beginning with colostrum and extending over all stages of milk secretion, with findings always in the negative. Human milk, on the contrary, contains surprisingly large amounts of diastase, in fact, far larger quantities than could be anticipated from data available in literature. According to Moro 100 cc. of milk are able to convert 25 gm. of starch into reducing matter in 24 hours. We have had 59 cases under our observation, of which nearly one-fifth produced more than 20 gm. of reducing matter in a single hour; this calculated for 24 hours means 480 gm., as against Moro's 25 gm. The most active milk in our series converted 52 gm. of starch into reducing sugar in one hour.

In the majority of the cases 100 cc. of milk formed from 6 to 15 gm. of reducing matter per hour. In one instance only was there a transitory absence of diastase; in several determinations in the course of 10 days following delivery the reducing sugar produced by 100 cc. of milk varied between zero and one gm. per hour. The blood diastase, which in others of our cases was normal, also showed an abnormally low level in this woman.

The values here reported are obtained under the standard conditions outlined above; we found, however, that the amount of sugar which milk diastase is able to produce is increased by 30%, if the temperature is raised to the optimum of about 53°, and another increase of about 50% is effected by adjusting the pH of the reaction mixture to the optimum of 6.8. This means that the most active milk in our series, which under our convenient standard conditions

[11] Somogyi, M., *J. Biol. Chem.,* 1930, **86**, 655.

[12] Somogyi, M., *J. Biol. Chem.,* 1931, **90**, 725.

[13] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.,* 1933, **100**, 695.

produced 52 gm. of reducing sugar per hour, would under the optimum conditions produce approximately 100 gm. of sugar in one hour.

The level of the diastase content is subject to continuous and rapid changes at the beginning of lactation. In the colostrum stage we find invariably higher diastase contents than in the advanced stages of lactation; about the 6th to the 8th day of lactation the diastase content attains a rather permanent level.